Forthcoming Articles

On Diabetic Acidosis A Detailed Study of Electrolyte Balances Following the Withdrawal and Reestablishment of Insulin Therapy D W Atchley R F Loeb D W Richards Jr, E M Benedict and M E Driscoll.

The Lipids of the Blood Plasma in Epilepsy I and II I McQuarrie, W R Bloor, C Husted and H A Patterson

Skin Reactions to Nucleoprotein of Streptococcus Scarlatinae in Patients with Rheumatoid Arthritis and Rheumatic Fever C S Keefer W K. Myers and T W Oppel.

Experimental Pneumococcus Lobar Pneumonia in the Dog I, II and III E. E Terrell, O H Robertson and L T Coggeshall.

Plasma Protein and Plasma Colloid Osmotic Pressure in Pathological Conditions with Special Reference to the Occurrence of Edema E Muntwyler C T Way, D Binns and V C Myers

The Calorigenetic Action of Thyroxin Polypeptide. W T Salter, J Lerman and J H Means

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STUDIES ON GASTRIC SECRETION

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In the voluminous literature on gastric function one traces the gradual destruction of the hope that gastric analysis can give exact diagnostic information The literature reveals instead, the enormous variability of functional activity of the normal stomach Achlorhydria has been observed frequently in persons who continue to be healthy and otherwise entirely normal In pernicious anemia achlorhydria is not always present, as the findings of Castle (1) and his associates indicate When so-called hyperacidity is found, it is often regarded as the cause of certain symptoms, but is as often present without symptoms Despite the fact that we cannot ordinarily establish a definite diagnosis on the basis of gastric analysis alone, it gives supportive information of unquestioned value

The fashion in test meals has, over a period of years, favored foods common to the usual dietary The idea has been that bread and water, milk, broth or even a full meal offered a physiological stimulus, and such meals as the Ewald, Boas, Sahli and Riegel have enjoyed the favor of many years In recent years a dilute solution of alcohol has gained vogue as a test substance because it is a clear solution and provokes a significant secretion of gastric juice In 1920, the action of histamine on gastric secretion was first studied by Popielski (2) In 1922 stimulation of gastric secretion by histamine was applied to man by Carnot, Koskowski and Libert (3) Since then it has been increasingly used in gastric analysis, largely because it was thought to give a more intense, possibly maximum stimulation

At the present time, the Ewald alcohol, and histamine stimuli are widely used in this country They have been extensively studied, but these studies have yielded very little information by means of which their actions can be adequately compared This is due to the fact that insufficient allowance has been made for the wide range of functional variation of normal gastric secretion In order to balance this variable in considering two test meals, it is necessary to give both of them to the same subject, under standard conditions and compare adequate series of such duplicate analyses With this in mind, a study of gastric function was undertaken A total of three hundred and fifty seven analyses were made on ninety-eight subjects under controlled conditions

acidity, which is in accord with the findings of Bloomfield and Keefer (4) and Comfort and Osterberg (7) The latter authors doubt the diagnostic value of volume estimation

II Comparison of Ewald and alcohol test meals, and histamine

The extensive use and study of these test substances has provided very little information by means of which their powers to stimulate the flow of gastric juice could be accurately compared In an attempt to do this, three series of duplicate analyses were made, using respectively the combinations alcohol and histamine, Ewald and histamine and alcohol and Ewald The arithmetical averages are given in Table I The coefficient of dispersion $\left(\frac{\text{average deviation}}{\text{mean}}\right)$ is used as the index of reliability The particular suitability of the index for the type of comparisons drawn in this study has been pointed out by Karl Pearson (21) The coefficient of dispersion was not calculated for volumes of secretion throughout, because these values showed no consistent trend nor was it calculated for the two cases in which gastric analysis was done following injection of calcium chloride

Histamine and alcohol In the first series, twenty seven subjects received both the alcohol meal and the histamine meal In all, sixty-one analyses were made under the standard conditions described previously Following histamine, twenty nine fractions contained no free hydrochloric acid, whereas after alcohol thirty nine fractions contained no free acid From the figures of Table I it will be noted that histamine elicits consistently higher values for both free and total acidities After histamine the acidity reaches a maximum at the end of thirty minutes falling off thereafter The rise in acidity after alcohol is more gradual and reaches a maximum at the end of an hour The volume of secretion is practically the same in both instances, although it is to be remembered that the alcohol test starts off with a gratuity of 50 cc

Histamine and Ewald Nineteen subjects were examined A total of forty five gastric analyses were made Seventeen specimens contained no free hydrochloric acid after the bread and water meal whereas after histamine the number without free acid was fifteen From Table I the following facts are evident The free acid after the Ewald test is significantly lower than after histamine The total acidity after histamine reaches a maximum at the end of forty five minutes, and exceeds that of the highest Ewald value by a small margin Both free and total Ewald acidities reach a maximum at the end of an hour Molinari Tosatti (8) found that the secretion after a similar test meal lasted longer than after histamine The volume of gastric contents is slightly higher after the bread meal The initial Ewald intake of 200 cc of water must be taken into consideration in drawing comparisons

TABLE I (*continued*)

Test substance	Number of tests	15 minutes					30 minutes					45 minutes					60 minutes				
		Volume	Free acid	Coefficient of dispersion	Total acid	Coefficient of dispersion	Volume	Free acid	Coefficient of dispersion	Total acid	Coefficient of dispersion	Volume	Free acid	Coefficient of dispersion	Total acid	Coefficient of dispersion	Volume	Free acid	Coefficient of dispersion	Total acid	Coefficient of dispersion
		cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.		cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.		cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.		cc.	cc. N/10 per 100 cc.		cc. N/10 per 100 cc.	
Ewald	12	15 7	14 0	0 76	45 7	0.30	15 5	25 0	0 54	56 5	0 35	13 0	36 1	0 34	69 5	0 18	15 9	36 6	0.39	69 4	0.25
Ewald	12	13 4	27 4	0 68	53 2	0.35	12 8	24 7	0.57	52 0	0 24	12 3	23 4	0 41	51 4	0 22	15 2	28 9	0 59	52 1	0.36
Histamine	11	10 6	30.3	0.55	55 7	0.32	11 9	42 5	0 48	64 7	0 34	10 6	44 9	0 45	70 2	0.39	11.3	39.5	0 49	59 4	0 39
Histamine-Atropin	11	10 3	36 4	0 53	61 1	0.35	12 3	47 4	0 42	69 7	0 30	10 8	47 4	0 45	70 2	0.30	9 1	45 1	0 47	67 2	0.32
With breakfast	9	10.3	13 9	0 99	56 4	0 47	10 2	26 9	0 99	64 4	0 54	8 6	32 1	0 80	74 2	0 90	9 1	33 4	0 85	72 7	0 52
Without breakfast	9	10 4	17 2	0 94	41 6	0.34	10 8	25 8	0 66	49 1	0 24	8.8	31 9	0 72	56 6	0 23	10.8	37 8	0 68	71.3	0 27
Calcium chloride	1	36 0	26 0		43 0		13 0	32 0		49 0		58 0	32 0		51 0		70 0	39 0		52 0	
Ewald	1	18 0	4 0		17 0		27 0	19 0		41 0		27 0	32 0		52 0		24 0	40 0		52 0	
Calcium chloride	1	40 0	87 0		97 0		12 0	72 0		78 0		26 0	96 0		104 0		25 0	95 0		101 0	
Histamine	1	30 0	100 0		105 0		39 0	73 0		82 0		15 0	22 0		22 0		6 0	0 0		10 0	

* For volume free acid and total acid, averages of the values secured from the number of tests indicated are given Volume indicates total quantity of gastric content aspirated

Alcohol and Ewald Thirty-four analyses were made using seventeen subjects After the Ewald test twenty-eight fractional tests contained no free acid, whereas after alcohol only eighteen samples were negative for free acid A study of Table I shows that under similar conditions the Ewald meal gives higher values for total acidity than the alcohol test This is in accord with the previous findings in which the histamine values were more nearly approached by the Ewald test than by the alcohol test The free acid values after the Ewald meal are again low The volumes of secretion show no significant trend

III Effects of repeated and prolonged stimulation of gastric secretion

In order to study the changes taking place in gastric secretion during repeated or prolonged stimulation, the following experiments were performed The subject was given one of the test substances and four aspirations were made, in the usual manner, at intervals of fifteen minutes Care was taken to drain completely the stomach with the last aspiration After a rest of fifteen minutes the same test substance was then repeated and a second gastric analysis performed A series of patients was studied by means of each of the three test stimulants alcohol, Ewald meal, and histamine In order to study further the secretory function during prolonged stimulation, three successive administrations of histamine were given at intervals of forty-five minutes, and the fractional samples of the analyses compared in a series of patients

We were interested in discovering (*a*) Whether repeated stimuli brought forth the same or a lessened response, in other words whether the phenomenon of fatigue could be demonstrated, (*b*) Whether there were any differences in the capacities of the test substances to elicit a renewed or prolonged secretion, (*c*) Whether the so called "maximal" stimulus of histamine left in its wake a pronounced fatigue or a refractive period, (*d*) Whether the Ewald meal, a so called "physiological" stimulus, evoked a pattern of response different from that caused by histamine and alcohol

Ewald tests Two successive Ewald meals were given as described above, to twelve subjects The averages represented in Table I show that the first test meal evokes the progressive increase in acidity noted previously In the first fraction after the second test meal the acidity is greater than in the corresponding fraction after the first test meal In the remaining fractions after the second test meal there is both an actual and comparative falling off of acidity In other words, after the second test meal there is a short interval of augmented acid output followed by a lessened ability to respond

Alcohol tests Two successive alcohol tests, as described, were given to twenty-five subjects A total of twenty-eight duplicate tests were made It will be noted from Table I that the maximum response appears

in the third specimen after the first test After the second administration of alcohol the acidity in the first two fractions is greater than in the corresponding fractions of the initial analysis In the remaining two fractions the acidity diminishes absolutely and relatively The curve is similar to that obtained from the Ewald meals, except that after alcohol the accumulative effect is slightly prolonged

Histamine tests *Two successive gastric analyses* were made after histamine, as in the manner previously described, on forty-one subjects who received in all forty six paired gastric analyses Examination of Table I shows that the average free and total acidity becomes maximum in the second specimen (one half hour after injection) in both tests The first two specimens of the second test are more acid than the corresponding fractions of the first test In addition it is noted that the peak of both analyses is attained in the second specimen of the second test The last two specimens are lower than the homologous samples of the first test In other words two successive injections of histamine give a cumulative effect, followed by a decline in secretory activity

In order to extend the observation described above, and to determine whether the cumulative effect is carried to a third stimulation, six subjects were given *three successive injections of histamine* at intervals of three quarters of an hour, fractions being collected every fifteen minutes as before The peak of acidity (Table I), after the first injection came, as before, in the half hour (second) specimen After the second injection of histamine the output of acid was augmented in all three specimens The maximum was attained in the second specimen followed by a definite falling off in the third specimen, which was nevertheless higher than the corresponding third specimen after the first injection After the third injection of histamine there was slight augmentation of acidity in the first specimen, but a drop thereafter

These experiments demonstrate that repeated stimuli increase the secretion of acid This holds for the Ewald and alcohol meals, as well as for histamine This does not seem to be a true arithmetical summation phenomenon When three successive stimuli are given, the augmentation of acidity after the third is less than after the second Following the initial increase in output of acid on repeated stimulation, there is a definite reduction suggestive of a fatigue phenomenon Ivy and Javois (9) and Lim (10) observed a diminution of response on successive administration of histamine in dogs, although the interval between injections (3 hours) was probably too long to provoke any additive effect Best and McHenry (6) state, "The gastric secretory mechanism affected by histamine is not easily completely fatigued, but the effects of an injection of the amine is greatly reduced by the recent previous administration of the substance" Polland (11), on the other hand, found that repeated

injections of histamine produced the same volume and acidity Further investigations of the laws governing gastric secretion would be desirable

IV The effect of atropine after histamine stimulation

Polland (11), found that atropine, after histamine, caused a marked decrease in volume, a rise in titrable acidity, and a fall in total acid in the gastric secretions Best and McHenry (6), state, "There is fairly general agreement that atropine does not prevent the action of histamine on the human gastric glands" Using eleven subjects, we have made similar studies After the administration of 1 milligram of histamine sulphate aspirations of four samples were made at 15 minute intervals After a rest of 15 minutes, 1 milligram of histamine sulphate and from gr 1/100 to gr 1/50 of atropine sulphate were given in the same syringe Four aspirations were made as before The results are represented in Table I The values for volume showed, as before, no definite trend The second injection gave in the first two specimens an augmentation of titrable acidity comparable to that which might have been expected if histamine alone had been given The titrable acidity of the third and fourth specimens, however, either equaled or exceeded that of the corresponding specimens of the first analysis This means that the addition of atropine prolonged the high level of titrable acidity, inhibiting the falling off which generally occurs in the last two specimens To this extent atropine augmented the titrable acidity as Polland had noted

V Influence of breakfast on gastric analysis

Inasmuch as patients occasionally misunderstand instruction and come to the clinic after they have had breakfast, it was thought desirable to study a group of nine subjects making duplicate gastric analyses, with and without breakfast It is interesting that some of the test meals recommended, such as the Riegel and Fischer meals, constitute practically a full meal It is admitted, of course, that the presence of the breakfast introduces an unknown variable, which, as a general rule, makes study of the gastric contents unsatisfactory To what extent a previous breakfast interferes with the proper interpretation of gastric analyses we were interested in knowing On one day the subject was examined, after having eaten a customary breakfast at the usual time The gastric contents were aspirated and the analyses, thereafter, conducted in the manner previously described, using one of the test substances On another day, the patient came in without breakfast, and the analyses were repeated using the same test substance The results given in Table I show that the volumes of secretion were in close agreement The free acid shows no constant trend, although, on the whole, it was slightly lower after a previous breakfast, due perhaps to the buffer action of the food After a previous breakfast the total acidity showed a distinctly

higher level in all samples This might possibly be due to the augmentative effect of repeated stimulation which has been pointed out above

Free acid values, then, are slightly lower and total acidities markedly higher after the subject has had a previous breakfast than when he is examined in the customary fasting condition

VI Effect of intravenous calcium chloride on gastric secretion

The recent interest in the use of intravenous calcium chloride to relieve smooth muscle spasm prompted us to investigate its effect on gastric secretion Two patients were given 10 cc of 5 per cent calcium chloride intravenously after which fractional samples of gastric secretion were removed every fifteen minutes for one hour The volume and acidity were then compared, in one case, with an ordinary histamine analysis, and in the other with an Ewald test

The results in Table I indicate that intravenous calcium chloride is followed by an increased gastric secretion which, in the two subjects studied, was comparable in volume and acidity to that produced in Ewald and histamine tests

COMMENT

The comparative merits of the various test meals and substances has been the subject of wide discussion The Ewald meal has been criticized because of the low acid values or even achlorhydria which it evokes in the normal stomach In this respect the present study shows that the alcohol test is no marked improvement The histamine test has been objected to on the ground that it introduces an unphysiological and unnatural stimulus In this connection, it is interesting that Ivy (12) has recently isolated from the gastric mucosa histamine sulphate which he identifies with gastrin In considering the virtues of the various test substances for gastric analysis, almost no regard has been given to the influence of the test substances on duodenal regurgitation and the rate of emptying of the stomach Neale and Klumpp (13) have shown that during the height of histamine activity gastric contents do not appear in the duodenum and pancreatic secretion into the duodenum is inhibited Lim, Matheson, and Schlapp (14), Osborne (15) and others agree that pancreatic secretion is decreased during histamine activity This explains why gastric aspirations after histamine are uniformly clear, colorless, and unstained with bile With the other test substances the ebb and flow through the pylorus introduces an uncontrolled variable which modifies both volume and acidity

The stomach shares with the kidneys, heart, lungs and other organs a wide range of functional capacity and a large margin of reserve Because of this a mild test stimulus comparable with the usual physiological functional demand, is unreliable because the reserve ability of the organ

will mask early functional impairment In order to detect such slight or early impairment, it is necessary to apply a maximal stimulus, a functional load which will try the organ to its full capacity It is our belief that this principle, which has been recognized and applied in the study of renal, pulmonary, pancreatic and cardiac disorders, should be considered in studying gastric function A stimulating agent should be used which is sufficiently intense to overcome the large margin of functional reserve that may mask the defect This concept has been previously expressed and advantageously applied in the study of disease by Bloomfield and Polland (16) It has been amply demonstrated that histamine provides a more intense stimulus than the other test substances, in adequate dosage, possibly a maximal stimulus This we believe is the most satisfactory single agent for the study of gastric function

The advantages of the histamine stimulus over the various test meals have been recognized by Bloomfield and Polland (16), Rudd (17), Carnot and Libert (18), Andresen (19) and others The following is a summary of some of these advantages

1 The variables of salivary dilution, appetite or anorexia, and rate of eating incidental to test meals are eliminated (15) 2 The gastric contents are uniformly clear, colorless and watery 3 A tube of small bore may, therefore, be used, making nasal passage possible, especially for children and uncooperative patients Such a tube and tip has been described by one of us (20) The stomach may be emptied at each fractional aspiration 5 The peak of secretory stimulation is attained within forty-five minutes, thus making the test shorter than when the Ewald and alcohol meals are used 6 The masking buffer action and reaction of the meal is not present 7 Histamine provides a strong stimulus The advantages of the functional load in gastric analyses has been discussed 8 The histamine test gives more nearly constant results on repeated examinations of the same individuals (15) 9 The histamine test is the only one that can be relied on for the detection of achlorhydria 10 During the height of histamine response neither duodenal regurgitation nor loss of gastric contents occurs (12)

SUMMARY

1 In a given individual the secretion of acid in response to a uniform stimulus tends in general to remain within a broad range Occasional wide fluctuations are, however, encountered

2 One negative histamine test is not conclusive evidence of achlorhydria

3 The volume of gastric secretion in response to a uniform stimulus fluctuates widely, and no relation between volume and acidity is found

4 In a series of duplicate analyses histamine elicited consistently higher values for free and total acid than the Ewald and alcohol tests

The maximum secretion of acid was attained between 30 and 45 minutes after the injection of histamine After the Ewald and alcohol tests the maximum response came at the end of an hour or later

5 The alcohol test gave higher values for free acid than the Ewald meal, but the latter evoked higher total acidities in duplicate analyses

6 In duplicate analyses, achlorhydria appeared more frequently after the Ewald meal than after alcohol, and least frequently after histamine

7 Administration of repeated gastric stimuli induced a temporary augmentation of acidity after each, followed by a pronounced falling off, suggestive of a fatigue phenomenon There was no fundamental difference in the power of the three test meals to elicit this response

8 Atropine in moderate doses tends to prolong the high level of titrable acidity which ordinarily occurs after histamine

9 A previous breakfast tends to cause lower acid values and higher total acidities in response to a standard stimulus

10 Intravenous calcium chloride increases gastric secretion

11 The theoretical and practical advantages of studying gastric function by means of the histamine stimulus are discussed

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STUDIES OF THE BASAL WORK AND OUTPUT OF THE HEART IN CLINICAL CONDITIONS

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Any method of determining the volume of the cardiac output in man which requires intelligent, alert and active cooperation by the subject can scarcely be regarded as suited to the study of this most important aspect of cardiac function in the clinic Errors are sure to arise from ignorance, lack of training, excitement, apprehension, etc., of such magnitude as to render results worthless When the ethyl iodide method of Henderson and Haggard (1) was devised, in which the sole requirement of the subject is that he breathe through a mouthpiece, it appeared that a long step had been taken toward the attainment of quantitative information concerning the heart's output under the various circumstances of disease Impressed by these possibilities Starr and Gamble, at the suggestion of Professor A N Richards and with generous assistance from Professor Henderson, learned to use the method and began the study of normal subjects in order to accumulate the experience and the data requisite for its profitable application to patients Unfortunately discrepancies were soon encountered between their experiences and those which Henderson and Haggard had published

It was soon evident that, by means of the analytical method of Henderson and Haggard, known concentrations of ethyl iodide could not be estimated with satisfactory accuracy It was therefore necessary to devise new methods which met this requirement (2) Employing these methods Starr and Gamble were unable to verify certain of the conceptions on which the original method was based, namely the absence of ethyl iodide from blood returning to the lungs and the size of the distribution coefficient for ethyl iodide between air and blood (3) They were therefore confronted with the question of abandoning the project altogether or of so altering the plan of the method that it should yield results in which they could have confidence The latter alternative was chosen, the distribution coefficient was redetermined and the method was redesigned so that the relatively large amount of ethyl iodide in venous blood could be estimated at each determination of cardiac output By means of this new method it was found possible to make satisfactory estimations of the flow of blood perfused through the lungs of dogs at a known rate

(4) Also numerous experiments on man gave evidence that it was proper to employ the method for the determination of cardiac output in resting subjects These modifications of the original ethyl iodide method and of the conceptions underlying it have recently been approved by Professor Henderson (5)

With these improvements duplicate determinations of the cardiac output of trained subjects yielded results which showed an average difference of only 6 per cent (6) On one subject under conditions of basal metabolism fifteen estimations have been made on seven days scattered over a period of four years There is no significant difference among these results, the most divergent being less than 9 per cent, the average deviation being 4 per cent from the mean of the series Although all subjects do not yield results as consistent as these, it seems proper to suppose that the cardiac output is relatively constant when conditions are suitable and that there exists a definite basal level of cardiac output analogous to the basal metabolic rate

The next task was to discover how best to secure a basal cardiac output in patients and to find the factors which, by increasing cardiac output, would prevent the attainment of this condition It was found that a longer rest period was necessary than that ordinarily employed before a determination of basal metabolic rate Cold, the taking of food, and excitement were found to increase cardiac output, and so must be avoided if the basal cardiac output is to be determined (6)

During this investigation other methods of measuring cardiac output were being developed and improved in other laboratories At present a number of methods give an average result on normal young adult males which is quite similar to that obtained by our method The diversity of the means employed in these methods, e g acetylene and a modified Krogh and Lindhard technique by Grollman (7), cardiac puncture and the Fick principle by Lauter (8), x-ray photography by Meek and Eyster (9), gives us confidence in the approximate correctness of our results Though some methods have certain advantages over our procedure, all but that of Meek and Eyster, which has certain disadvantages, either disturb the subject by puncturing the skin or by requiring his cooperation, and so appear to us less suitable for use on untrained patients than the procedure we employ

Therefore it appeared that the time had come to carry out the original intention of studying cardiac output in the clinic and to attempt to answer the question whether the method of Starr and Gamble was sufficiently accurate to throw light on the diagnosis and treatment of cardiac disease and on other questions of cardiac physiology Accordingly, duplicate determinations of basal cardiac output were made in various types of patients About twenty-five cases had been tested before the data disclosed that in estimating the condition of the heart, the basal cardiac

output was of less significance than the cardiac work. The results also indicated that the basal work of the heart was a function of its size a finding which suggested that Starling s Law of the Heart was applicable to clinical conditions Therefore, we decided to attempt to estimate the magnitude of the heart's work in the common clinical conditions in which our method could be properly utilized

We have now assembled duplicate determinations on 50 persons who were lying at rest fifteen or more hours after the last meal Seeking for diverse conditions we have studied cases whose pulse rate ranged from 46 to 142, the blood pressure from 94/50 to 210/130, the cardiac output from 1 3 to 7 5 liters per minute, and the cardiac silhouette area from 69 to 200 cm^2 Among these cases are apparently normal persons patients threatened with congestive failure, and patients with some circulatory abnormality but not threatened with failure, i e hypertension, hyperthyroidism, angina pectoris, anemia, compensated valvular heart disease and functional heart disease In cases not threatened with congestive failure the relationship between the left ventricular work per beat and the area of the cardiac silhouette or the volume of the heart is approximately linear, and the deviation of the great majority of values from the best line is within the limits of error Values obtained from patients threatened with failure are significantly different from these They demonstrate inefficient hearts, doing but little work in proportion to their size This affords confirmation of the classic conception of cardiac failure and may afford an additional means of diagnosis of this condition

METHODS

The details of our method have been described (4) The subjects during each respiratory cycle inhale a concentration of ethyl iodide which causes no discomfort being indeed not much more than perceptible After about 20 minutes of such inhalation, samples are taken of inspired expired, alveolar and rebreathed air A second group of samples is taken about 10 minutes later The difference of ethyl iodide content in inspired and expired air multiplied by the volume of respiration gives the amount of this gas absorbed from the lungs The difference between the contents of alveolar and rebreathed air, in tension equilibrium with arterial and mixed venous bloods respectively multiplied by the distribution coefficient of ethyl iodide between air and blood, gives the amount of the gas carried away by each unit of blood The quotient of these two items gives the amount of blood which flows through the lungs, i e the cardiac output In calculating the results recorded in Table II we have omitted one of the small corrections previously employed, that for the change of ethyl iodide in rebreathed air with time This correction has proved to be insignificant in determinations of this kind

In the great majority of cases we employed the average distribution coefficient of ethyl iodide between air and blood, 6 1, which when corrected to the usual room temperature and vapor tension became 5 6 (4) In cases of anemia and of diabetes this coefficient was determined and the result utilized The volume of respiration during the period in which the samples were taken, usually 2½ minutes, was employed in the calculation Blood pressures were determined by the auscultatory method, and pulse rates were counted during this same period

The metabolism was determined by slowly drawing samples of expired air from the mixing bottle during the period in which the cardiac output samples were taken A second estimation of metabolism coincided with the second cardiac output determination Thus the same volume of respiration was used for calculating both metabolism and cardiac output, and the samples used in both determinations were taken as nearly simultaneously as technical considerations would permit The analyses were done in duplicate in the large majority of instances The respiratory quotient employed was that determined except in the few cases in which it was altered by obvious hyperventilation, when 0 82 was assumed The standards used were those of DuBois

The size of the heart was determined by orthodiagraph with the patient standing The anteroposterior silhouette was drawn at the end of a normal inspiration and its area computed by a planimeter Many of these determinations were done immediately after that of the cardiac output and metabolism All those on patients were done within a few days However in seven normal persons (numbers 43 to 49), whose basal cardiac output had been determined in a previous investigation (6), the orthodiagrams were made about a year later

The analyses pertaining to cardiac output were almost entirely performed by Starr, those concerned with metabolism by Collins and the majority of orthodiagrams were made by Wood We are indebted to Dr Alexander Margolies for the remainder of the orthodiagrams The person determining the heart size was never aware of the other findings

On the avoidance, detection, or evaluation of certain errors

We have not omitted any result from Table IV or Figure 1 However, certain considerations make some of doubtful validity

(*1*) *Failure to attain the basal condition* Aside from conditions in which the metabolism was elevated by disease, thyrotoxicosis and some cases of threatened congestive failure, only two of our cases (numbers 9 and 11) failed to attain the basal condition in at least one of the two estimations Five cases attained it in only one of the duplicates, but when the average of the two is within normal limits we do not regard the deviation of one as significant. Therefore we have evidence that most of

our subjects were under no emotional strain when their cardiac output was estimated and we attribute this to the fact that no cooperation was required But we believe that under certain circumstances it is possible to have a basal metabolic rate without a basal cardiac output For this reason our preliminary rest period was always longer than that ordinarily used to secure a basal metabolic rate

(2) *Uncertainty concerning tension equilibrium between alveolar air and arterial blood* This equilibrium, which has been demonstrated for ethyl iodide when the lungs are normal (4), cannot be assumed to exist when the lungs are abnormal No patient whose lungs were manifestly abnormal has been included in our series The cases of "threatened congestive failure" were persons who had been decompensated but had regained compensation under treatment, and in whom abnormal pulmonary physical signs could no longer be detected None of them showed any cyanosis In the case which appeared to us closest to actual decompensation (number 26) the oxygen saturation of the arterial blood was 89 per cent, just outside normal limits We do not believe that the pathologic change in the lungs would cause significant error in this case, for the more soluble ethyl iodide may be expected to pass through the lungs with more facility than oxygen Cases having emphysema were avoided

(3) *Hyperventilation* The fact that certain patients overventilate and blow off their CO_2 when breathing through apparatus is well known We have encountered this type One patient (number 1 and 2) showed this phenomenon only on certain occasions This gave us the opportunity to determine its effect on the cardiac output and work. The results (Table IV) show that when respiration was elevated, basal cardiac output and work were usually exceeded When the respiration was normal the cardiac output and work were at a minimum and reasonably consistent. Therefore hyperventilation is a valid cause for discarding a result The results charted for this patient have been selected from those in which the respiration was not unduly elevated When other patients hyperventilated (cf numbers 12 and 30) the results have been charted but will be commented on below

It is noteworthy that the alveolar CO_2 of our untrained subjects at the end of the breathing period averaged only 4 73 per cent as against 5 34 per cent for our trained subjects This reduction was plainly due to overbreathing in a few of our subjects and may well have been influenced by acidosis in some others That untrained subjects, breathing through metabolism apparatus, usually blow off CO_2 has been demonstrated by Heckscher (10) and we believe that this factor has entered into our results also The low figures are not to be attributed to any error in our method of obtaining alveolar air, for Haldane Priestley samples taken on a number of cases contained amounts of CO_2 of about the same magnitude

(4) *Errors inherent in the estimation of cardiac work* The work of the heart may be calculated from the familiar formula (11)

$$W = QR + \frac{wV^2}{2g},$$

where W = work done per minute or per beat, Q = volume of blood expelled per minute or per beat, R = arterial resistance = mean arterial pressure in mm Hg $\times$ 13 6, V = velocity of blood at aorta, w = weight of blood, g = acceleration due to gravity

The expression $wV^2/2g$ represents the work done in imparting velocity to the blood To estimate it, one would have to make certain assumptions Assuming that the aortic diameter is 2 8 cm , that systole is proportional to the square root of the cardiac cycle (12) and that the expulsion period equals systole minus 0 06 second we have calculated the velocity factor for the four cases having the largest cardiac outputs and highest pulse rates In no case did this factor represent more than 2 per cent of the calculated cardiac work. As this factor would certainly be smaller in the other determinations we have omitted it from our calculations altogether

The error inherent in our cardiac output determinations has been discussed [1] (6) Unfortunately, there is no means of accurately evaluat-

[1] Lindhard (33) makes the statement that the maximal error inherent in the estimation of ethyl iodide in 500 cc of air by our method is 0 3 mgm This is erroneous, the context suggests that it is a misprint for 0 03 mgm , the average error in the analysis of a single sample But the following statement that this error induces an error of 6 to 7 per cent in the result of each of the four samples of a cardiac output estimation is likewise incorrect This percentage is applicable to the smallest of the factors utilized to calculate cardiac output (4) In this same article the description of our technique of obtaining air in equilibrium with venous blood is also incorrect Our subjects rebreathe for 30 seconds in and out of a bronze bag and not for 2 minutes in a Douglas bag as there stated

In this investigation the smallest factor appearing in the calculation of cardiac output (the alveolar ethyl iodide concentration minus the rebreathed concentration) was usually much larger than in previous experiments and the influence of the error inherent in the analysis on the calculated cardiac output is correspondingly smaller

It must be remembered that, when the accuracy of our analytical method was evaluated by the estimation of known amounts of ethyl iodide, the apparent error was affected by that inherent in the weighing and handling of such small quantities Also in the calculation of cardiac output the values obtained by analysis are subtracted one from another, and they appear in both the numerator and denominator of the equation Therefore any errors which changed these values by a like amount, as those caused by constant under- or overtitration, would not affect the calculated cardiac output. Similarly, any error which affected all four values proportionately would also cancel out These reasons afford an explanation of the fact that the duplicate estimations of cardiac output on trained subjects agree better than one might expect from our accuracy in estimating weighed amounts of ethyl iodide

ing the absolute error. The average deviation of the forty seven duplicates from their mean is 6 per cent. The standard deviation is 7.3 per cent. Therefore differences of 20 per cent are likely to be significant. The differences between duplicate estimations in trained and untrained subjects are given in Table I and average about 6 per cent and 11 per cent.

TABLE I

Average difference between results of duplicate determinations in per cent of their mean

	Respiration, 2½ minute period	Metabolism, 2½ minute period	Cardiac output per minute	Cardiac work per beat
	per cent	*per cent*	*per cent*	*per cent*
In 9 trained subjects (normals)	2.8		6.5	6.5
In 44 untrained subjects (hospital patients)	7.2	9.9	11.4	10.8

of the corresponding means. These differences are of about the same magnitude as those found in measuring metabolism [2] and not much larger than those observed in measuring respiratory minute volume. In ten subjects the change in respiration will almost completely account for the difference between the duplicate cardiac outputs. Trained subjects are more consistent than untrained ones in every item.

We regard automatic sampling of alveolar air as the chief hazard in our method. The respiratory tracing permits the detection of the shallow or irregular types of breathing which would invalidate this method. We have encountered very few of these and we have made no eliminations on this score. Untrained subjects usually breathe fairly deeply through apparatus. Also in most of our patients the arteriovenous ethyl iodide difference is large. Therefore small errors due to faulty automatic sampling would make but little difference in the calculated cardiac output.

In calculating arterial resistance we have made the usual assumption that the mean of systolic and diastolic pressures equals the integral of the pressure curve. Starling (13) evaluated the maximum error of this assumption at 10 per cent., Frank (14) also regarded the error introduced as 10 per cent. but exceptionally it might be much higher (15). In our series it would probably be largest in the cases of hyperthyroidism (numbers 11, 21, 22, 23, and 28) and in the one case with aortic regurgitation (number 24). The one case of auricular fibrillation in the series was under the influence of digitalis and there was no difficulty in estimating

[2] It is to be remembered that the metabolism determinations were for short periods usually 2½ minutes to make them coincide exactly with the cardiac output determinations. The deviation of the duplicates is therefore considerably larger than is usual in determinations made over longer periods of time.

blood pressure We believe that the error would be large only in unusual conditions and so, considering our series as a whole, we have estimated the error involved at 10 per cent We are by no means certain that the usual method of estimating diastolic pressure gives the correct result, but this error would be largely systematic, and we lack the data necessary to assign a value to it

(5) *The error in estimating cardiac size* To determine the personal error in drawing the silhouette ten patients were studied by Dr Wood and Dr Margolies independently The average difference between the silhouette areas of each patient was 5 9 per cent of the smaller value, the maximum difference was 13 per cent The largest absolute differences were in patients with large hearts, the shadow of the lower border being lost in the diaphragmatic shadow The largest percentile differences were in patients with very small hearts

The error in estimating cardiac volume from the silhouette was studied by Bardeen By applying a formula, heart volume $= 0\ 53A^{3/2}$, where A was the area of the silhouette in the anteroposterior position in cm^2, he estimated the heart volume of 63 cadavers In 45 cases the error was less than 10 per cent The error in the living should be less, for movement aids greatly in the identification of the cardiac border Bardeen believed it to be less than 5 per cent in the majority of instances (16)

Certain factors might make the cardiac silhouette area bear an inconstant relation to the volume of the heart In some hearts the anteroposterior diameter bears an unusual relationship to the transverse diameter Also in large hearts the chambers do not always enlarge proportionately Especially in mitral stenosis the percentage of cardiac area contributed by the left ventricle may be smaller than in normal hearts or in hearts whose enlargement is due to other lesions

Therefore it seemed to us that our error was larger than that suggested by Bardeen We have allowed $\pm$ 10 per cent for this error, but under exceptional circumstances it might be much larger than this

Our data permit us to estimate the work of the left ventricle There is evidence that the work of the right ventricle bears a constant relationship to this (17) We prefer to avoid the assumptions necessary to calculate the work of the whole heart In our discussion only the left ventricular work will be considered

In discussing the relations of the volume of the heart, demonstrated to be approximately equal to 0 53 $area^{3/2}$ by Bardeen, we have omitted the constant (0 53) for the sake of simplicity

On the statistical methods and terminology used in the analysis of the data[2]

For brevity in the description of our data, we have made use of the ordinary statistical terminology. As these terms are not yet common in clinical literature it is proper to indicate their meaning. The *mean* needs no explanation. The *standard deviation* is a measure of the deviation of the individual values from the mean of the group. In most of our charts the points appear to have arranged themselves about a straight line. The line which fits the points best can be calculated and is called the *best line* or the *regression line*. There are two such lines for most groups of values but only one of them, the regression of cardiac work on heart size, deserves consideration in our discussion. The *standard deviation about a regression line* is a measure of the deviation of the points from this line. The *correlation coefficient* is likewise a measure of this deviation. A coefficient of plus or minus 1.00 indicates perfect correlation, all the points being on a line. A coefficient of 0 indicates no correlation, the points being scattered without regard to any line.

A summary of the statistical analysis is given in Tables II and III, and statistical methods have been employed in placing lines on certain of the figures. The solid line AB represents the best line, the regression of heart work on heart size, for the control group. On either side of AB, at a distance equal to twice the standard deviation about the regression line for the control group, the broken lines CD and EF have been placed. These lines should enclose approximately 95 per cent of all similar "control" cases. Accordingly only 5 per cent of such cases would lie outside the enclosed area, and therefore the probability is about 97.5 in 100 that any result falling to the right of this area was secured in a case which was abnormal in respect to the relationship illustrated by the chart.

It is of some interest to compare the limits of the control group based on its statistical distribution with our estimates of the errors involved in our determinations as discussed before. Allowing 20 per cent, 10 per cent and 10 per cent for the errors in the estimations of cardiac output, mean blood pressure and heart size respectively, we have constructed on Figure 1 the lines GH and KL which would include in the area between them all the points whose deviation from the best line AB could be explained by the errors which we believe to be inherent in these estimations. Almost all of the results obtained on the control group fall within this area; those obtained on the cardiac group are all outside it. However, since we can never hope to obtain an accurate estimate of the size of our absolute errors, the statistical methods afford the better means of determining significant deviations from the normal.

[2] We are indebted to Dr. W. C. Stadie for instruction and assistance in the statistical analysis of the data.

RESULTS

The results are given in detail in Table IV, which has been placed at the end of this paper For the purpose of study and analysis the cases have been divided into several groups

Group I consists of six cases of cardiac disease, who, though now compensated, at one time had congestive failure Therefore there can be no doubt that they had serious myocardial disease They are shown on the charts (Figures 1, 2, 3 and 5) as squares and in Table IV, numbers 24 to 29

Group II is composed of seventeen cases of hypertension whose cardiac condition appeared to be satisfactory (Figures 1 to 5, Table IV, numbers 1 to 17)

Group III is a mixed group of 22 cases including normal persons, cases of hyperthyroidism, anemia, functional heart disease, well compensated valvular heart disease, and two cases of angina pectoris (Figures 1, 2, 3, 5 and Table IV, numbers 19, 21, 30 to 49)

The control group consists of Groups II and III considered together, and stands in contrast to the cardiac group The cases in Groups II and III are united by the fact that none of them has ever had congestive failure nor did any appear to be immediately threatened with it The union of these two groups represents an attempt to gather together cases with normal myocardia, subjected to different types of strain The cases of angina pectoris are included in it because it was found that they did not differ significantly from the rest of the group All these cases have been placed in the figures as circles

The propriety of combining Groups II and III into a single group has other support In the relationship in which we are most interested, that of cardiac work and heart volume, the statistical criteria indicate that there is a well marked degree of correlation in both groups and that the regression lines are not significantly different for the two groups Therefore, it is proper to combine them and this gives both an increased number of cases and a greatly increased range of cardiac size But in numerous other relationships as in Table III, item 2, the two groups are significantly different. Therefore, in the discussion of these relationships they will be considered separately and the data given in Tables II and III show both the relationships of the combined group and that of its two component groups

Four borderline cases could not be properly included in the above groups Two cases of severe hyperthyroidism, numbers 22 and 23, showed symptoms suggestive of threatened myocardial failure but had never actually become decompensated Number 20 was a case of severe gastric hemorrhage who was in shock when tested In Case 50, one pair of determinations was made during an attack of paroxysmal tachycardia The results on these four cases have been placed on the figures, the first three as circles, the last as a square. But they have not been included in the statistical analysis

In three cases estimations were unsatisfactory because basal conditions were not attained Case 32 was a young woman who had the symptoms, appearance

and pulse rate which suggested hyperthyroidism, but her basal metabolic rate had never been abnormal. Her excitement during the determinations manifested itself by a larger increase in cardiac output than in metabolic rate. Case 12 hyperventilated, the alveolar CO_2 falling to 2 per cent. Just after the first estimation on Case 9, accidental micturition excited the patient and the great increase in cardiac work which resulted illustrates the strain which emotion may put on the heart of a patient with hypertension. These results have been omitted from the statistical analysis. They have been charted in Figure 1 but have been omitted from the other figures.

The chief interest in our results lies in the consideration of the groups of cases. We selected these cases from diverse conditions in the belief that any relationship which held for all might be expected to be fundamental. That such a relationship exists for the control group is illustrated in the figures and they will now be discussed in detail.

Figure 1 shows the relationship between the left ventricular work per beat and the area of the cardiac silhouette. Each of the duplicate determinations has been charted and no results have been omitted. It will be seen at once that the great majority of circles representing the control group are arranged about the line AB while the squares representing the cardiac group are far distant. The lines CD and EF define limits of the control group selected on the basis of statistical analysis. The lines GH and KL limit the deviations from AB which could be accounted for by our estimates, by other than statistical criteria, of the errors inherent in our methods. Both these sets of lines enclose most of the circles in the area between them. Indeed the position of most of those outlying on the upper side may be explained by failure to attain the basal condition. Those outlying on the lower side represent cases whose hearts may not be normal. The squares lie well outside these areas. The deviation of the values of the control group from the line AB may be explained as due to the errors to which our procedure is subject, but the position of the values of the cardiac group cannot be so explained. Therefore our results show that there is a definite relationship between the heart's area and the basal cardiac work per beat in the cases not threatened with failure, whereas in cardiac cases this relationship does not hold.

Figure 2 differs from Figure 1 in that cardiac volume has been substituted for cardiac area on the horizontal coordinate. The values shown are averages of the duplicate determinations, and the unsatisfactory results on the three cases mentioned above have been omitted. The results again show the relationship between heart size and basal cardiac work per beat which exists in the control group, but does not hold for the cardiac group.

Figure 3 differs from the preceding figure in that left ventricular work *per minute* has been substituted for work *per beat* on the vertical coordinate. Though the relationship of heart size and work still holds for the

control group it is not so close and there is more overlapping with the cardiac group than in Figures 1 and 2

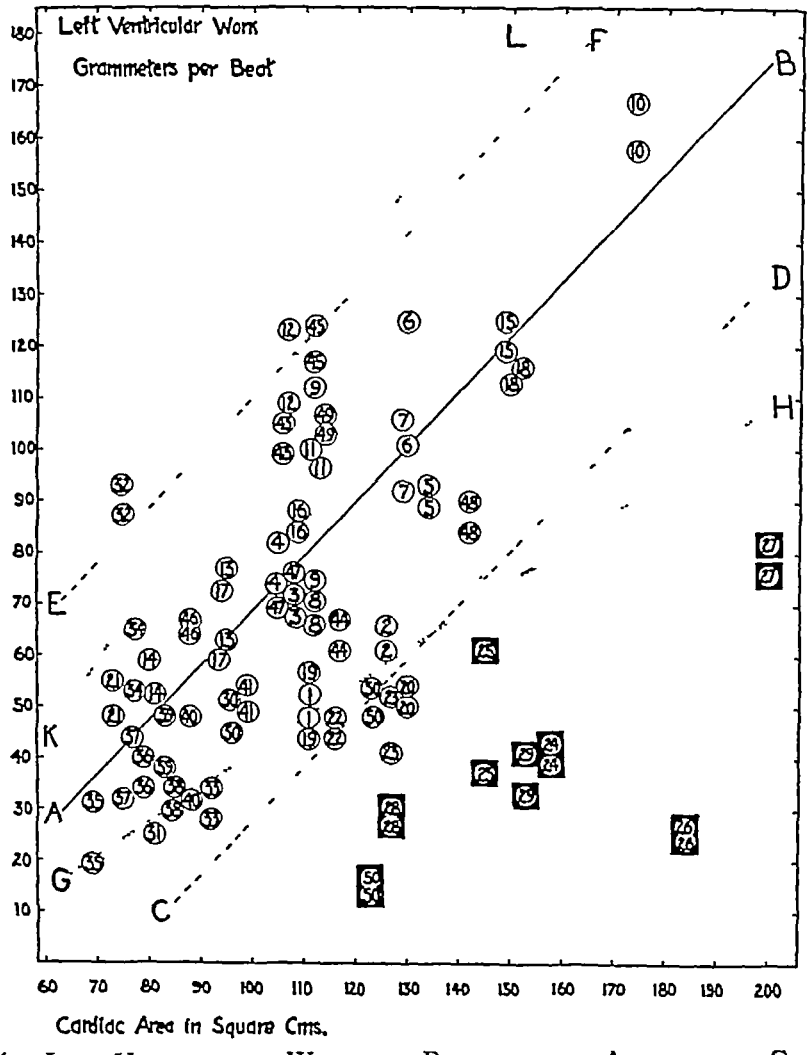

FIG 1 LEFT VENTRICULAR WORK PER BEAT AND THE AREA OF THE CARDIAC SILHOUETTE

Each of the duplicate determinations has been charted The numbers refer to the cases as recorded in Table IV The values for the cardiac group (Group I), and the case of paroxysmal tachycardia in an attack, are recorded as squares, the remainder as circles No cases have been omitted

The line AB represents the best line for the control group of cases, the regression of the work on the area Lines CD and EF have been placed at a distance of twice the standard deviation from AB, and define the approximate limits of this group as determined by statistical methods On the other hand, the lines GH and KL limit the deviations from AB which could be accounted for by our estimates, by other than statistical criteria, of the errors inherent in our methods

Figure 4 has the same coordinates as Figure 2 but only the cases of hypertension have been charted This group is of especial interest because of the great differences of heart size which occur within it. In spite of these differences the relationship between heart volume and heart work per beat is very striking and the figure has been given to illustrate this

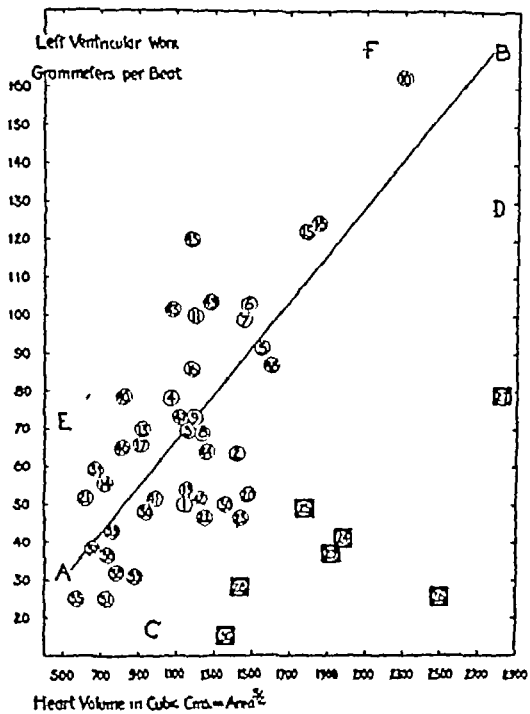

FIG 2 LEFT VENTRICULAR WORK PER BEAT AND CARDIAC VOLUME

The values charted are the average of duplicate estimations The unsatisfactory results on three cases have been omitted Lines and symbols as in Fig 1

Figure 5 has been given for the sake of contrast with the other figures It has been customary to report the results obtained by cardiac output methods in terms of output per minute per square meter of body surface Figure 5 shows the almost complete lack of relationship between basal cardiac output per minute and body surface in our patients This will be discussed later

A summary of the statistical analysis is given in Tables II and III The former shows the means of the more important observations together with the standard deviation of the values from their mean Table III

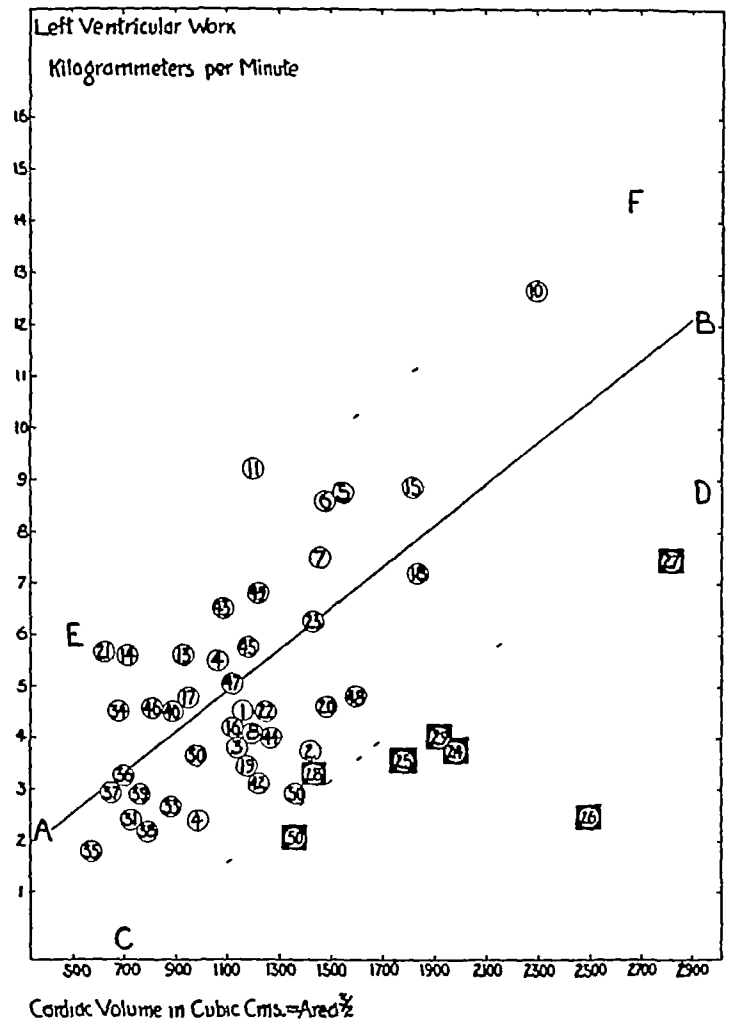

FIG 3 LEFT VENTRICULAR WORK PER MINUTE AND CARDIAC VOLUME

The values charted are the average of duplicate estimations The unsatisfactory results on three cases have been omitted Lines and symbols as in Fig 1

gives the correlation coefficients which pertain to the more interesting relationships

A more detailed account of the results and their significance will be postponed until the general discussion of the problems before us

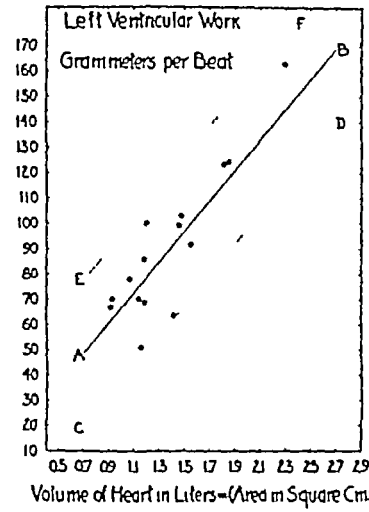

FIG. 4. LEFT VENTRICULAR WORK PER BEAT AND CARDIAC VOLUME IN 17 CASES OF HYPERTENSION (GROUP II)

The lines pertain to the values charted

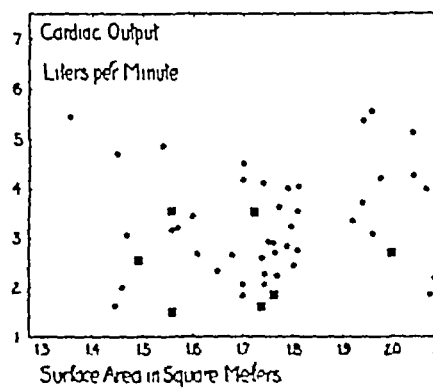

FIG. 5. CARDIAC OUTPUT PER MINUTE AND BODY SURFACE AREA

Symbols as in Fig. 1. No cases have been omitted. The values charted are the average of duplicate estimations

TABLE II

Means, with standard deviations from the mean

	Group II 17 cases Hypertension group		Group III 22 cases Mixed group		Groups II + III 39 cases Control group		Group I 6 cases Cardiac group	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
rdiac output, *liters per minute*	3 5	1 0	3 3	1 0	3 4	1 1	2 6	0 7
rdiac output, *liters per minute per sq meter body surface*	1 9	0 7	1 8	0 6	1 9	0 5	1 6	0 5
rdiac output, *cc per beat*	49	13	47	18	48	16	28	8
rdiac work, *kgm -m per minute*	6 6	2 4	3 9	1 4	5 1	2 3	3 8	1 5
rdiac work, *gram-m per beat*	90	27	57	27	72	32	42	17
rdiac work, *per beat per cm² cardiac area*	67	9	59	22	64	19	26	12
rdiac work, *per beat per liter heart volume*	6 7	1 0	6 1	2 1	6 4	1 7	2 1	0 6
rdiac work, *per minute per liter heart volume*	4 9	1 2	4 2	1 6	4 5	1 7	2 4	0 8
rdiac area, cm²	120	23	97	19	107	23	143	33
rdiac volume $= \frac{(\text{area in cm}^2)^{3/2}}{1000}$	1 38	0 44	1 00	0 28	1 16	0 40	1 78	0 42
ygen unsaturation of venous blood, *per cent of normal arterial saturation*	41	9	41	12	41	10	61	10
stolic pressure, *mm Hg*	174	25	110	8	140	36	150	40

TABLE III

Correlation coefficients

	Group II 17 cases. Hypertension group	Group III 22 cases. Mixed group	Groups II + III 39 cases. Control group
1 Level of significant correlation (for $P = 0.05$)*	0.48	0.43	0.41
2 Cardiac output per minute and body surface area	0.00	0.48	0.25
3 Cardiac output per minute and cardiac area	0.57	0.23	0.43
4 Cardiac output per minute and cardiac volume	0.77	0.42	0.59
5 Cardiac output per beat and cardiac volume	0.91	0.52	0.51
6 Cardiac output per minute and oxygen consumption	0.75	0.78	0.65
7 Cardiac output per minute and volume of respiration per minute			0.31
8 Cardiac output per minute and systolic pressure	0.34	0.57	0.29
9 Cardiac work per minute and cardiac area	0.64	0.34	0.69
10 Cardiac work per beat and cardiac area	0.85	0.53	0.77
11 Cardiac work per minute and cardiac volume	0.73	0.48	0.69
12 Cardiac work per beat and cardiac volume	0.85	0.48	0.76

* Since the level of significant correlation varies with the number of cases we have accepted the approximations to significant levels given by Fisher (31), Table 5A

Discussion

The work of the heart and its relationships

Starling's "Law of the Heart" and its extension to clinical conditions

The direct relationship between heart size and heart work, in conditions in which the heart was functioning well, was demonstrated by Starling in experiments on the heart lung preparation The principle is summarized in his Linacre Lecture (32) on the "Law of the Heart' There he speaks of the work of the heart as a function of its volume, later, as a function of the active surface of the muscle fibres, and again, as a function of the length of the muscle fibres In a later paper (18) a figure (number 5) shows that the relationship between diastolic ventricular volume and work per hour is linear This has also been shown to be true of work per beat (15)

Our findings are in complete accord with this hypothesis Figures 2 and 3 show that a similar relationship exists in the control group between

the work of the left ventricle per beat and per minute and the cardiac volume The correlation coefficients are 0 76 and 0 69 (Table III, items 12, 11) By assuming the pulmonary pressure to be one-sixth of the aortic pressure the relationship of the total cardiac work to the volume of the heart can be calculated but the correlation coefficients would not differ significantly from those given above Therefore we regard these high correlations as a demonstration that Starling's "Law of the Heart" may be extended to clinical conditions

Considerations involved in the employment of Starling's law to detect cardiac disease The results recorded in Figures 1, 2 and 3 show that in the clinic as in the heart-lung preparation the normal relationship does not hold for hearts which are threatened with failure This suggests that we have a means of detecting the hearts so threatened To attempt this it would be necessary to define the normal relationship between heart size and heart work, and especially to determine its limits, so that one could tell whether the results of a given case fall within or without the normal [or abnormal] group

Which of the relationships between the various aspects of heart size and heart work would be best for this purpose cannot be finally determined Those illustrated in Figures 1 and 2, heart area and heart volume against left ventricular work per beat, seem most promising In each of these the control group has a high correlation coefficient, 0 77 and 0 76 respectively (Table III, 10, 12) The lines representing twice the standard deviation include our values for the control group and exclude those for the cardiac group in both instances In favor of the latter relationship is the expectation that the volume of the heart would be more closely related to the mass of cardiac muscle, and therefore a better function for comparison with the heart's work, than the silhouette area But any estimate of the volume of the heart from the three halves power of the area involves an assumption which is not always valid, for the anteroposterior diameter of the heart is known to vary independently of the silhouette area in some instances By using the area this assumption is avoided On the other hand when the relationship between heart work and area is studied it is found that the control group has lost its unity The cases of hypertension form a group distinct from the remainder, the slopes of the respective regression lines are significantly different. When the cardiac work is plotted against the heart volume there is no such difference, so this relationship is to be preferred

The mathematical definition of the normal heart size-work relationship and the detection of abnormality by means of it The question of the normality of a given case could be answered by finding the position on Figures 1, 2, 3 or 4 of the point obtained by plotting the results, and determining whether it fell within or without the normal area bounded by the dotted lines *CD* and *EF* The same answer could also be obtained

by calculation from the equations of the regression lines, the normal limits being given by twice the standard deviation A third method might be found simpler by persons not accustomed to mathematical calculations The lower limit of normality may be given by the equations for the lower limiting lines (*CD*) For example

(1) 0 061 (cardiac silhouette area in sq cm.)$^{3/2}$—(L V work per beat in gm meters) = 42

Simplifying the following equation by rounding out the slope from 1 06 to 1 00,

(2) (cardiac silhouette area in sq cm)—(L V work per beat in gm meters) = 78

Thus if the values found in a given case are inserted in either of these equations and the result is greater than the figure given the probability is about 97 5 in 100 that the case is abnormal with respect to myocardial function

The limitations of this means of detecting cardiac abnormality This definition of the normal relationship between heart work and size on the basis of a group of diverse cases united only by the absence of congestive failure must not be considered as final Our results provide evidence that in one group, the cases of hypertension, it would be possible to apply more rigid criteria Figure 4 shows the relationship between work per beat and volume in the seventeen cases of this group The correlation is extraordinarily good, the coefficient being 0 85 (Table III, 10) The standard deviation about the regression line is so small that the limits of normality can be more closely defined than in the entire control group Therefore, a slight diminution in the heart work size ratio would place a case of hypertension outside the normal zone These results suggest that the statistics of other groups of cases, i e thyrotoxicosis, rheumatic heart disease, etc., might allow a more rigid definition of the normal and so permit detection of myocardial abnormality earlier in the course of disease This question cannot be answered till more data have been secured

On the other hand it is not to be expected that the relationship of basal cardiac work and size would permit detection of all types of cardiac abnormality, even if the methods should be greatly improved The result obtained in one case of angina pectoris (number 31) who later died of coronary thrombosis, is near the center of the normal range Another case of this disease (number 2) also gave results within the normal limits Those obtained on the third (number 30) are likewise normal but the examination was unsatisfactory because the patient hyperventilated So we find that the condition of the heart in these patients is different from that of the cases threatened with decompensation This finding is consistent with the diverse clinical courses of these two types of cardiac disease

The cardiac output and its relationships

To consider the relationships involving the heart's *work* is to concentrate attention on the condition of that organ But the cardiac *output* is of importance to the rest of the body, because the blood supply of tissues other than the heart (19) depends on its magnitude Therefore, in relation to metabolism and body size cardiac output is of chief interest, but in relation to heart size the output is of less interest than is the heart's work

Cardiac output and cardiac size In the control group the cardiac output is significantly related to the size of the heart, but the correlation is not as good as in the corresponding work relationships, except in the hypertensive group (Table III, 3 to 5, 9 to 12) This scattering of normal results interferes with the detection of cardiac abnormality by this relationship

Cardiac output and body surface area Our estimates of the cardiac output per minute per square meter of body surface, the usual method of reporting results, are shown on Figure 5 There is complete lack of correlation among the cases of hypertension The mixed group shows a correlation which is just significant A factor in this lack of relationship may be the close correlation between cardiac output and metabolism in both Groups II and III When the metabolism deviates from the expected basal level in disease a corresponding change in cardiac output usually takes place, so that the normal relationship of cardiac output to body surface is lost

The average cardiac output per minute, and this value per square meter of body surface (Table II, 1, 2) are both lower in the cardiac than in the control group but we have not yet obtained sufficient evidence to determine whether the difference is significant Certain patients with advanced myocardial disease have larger cardiac outputs than some normal persons Therefore, these values throw little light on the condition of the heart

Cardiac output and oxygen consumption Of more interest is the relationship between cardiac output and oxygen consumption, stressed by previous investigators as a result of studies on normal persons at exercise (20) and at rest (7) In our control group the correlation is good, the coefficient being 0 65 (Table III, 6), when Groups II and III are considered separately it is even better From this relationship the arteriovenous oxygen difference can be calculated In the cardiac group the amount of the arterial oxygen taken up by the tissues is 61 per cent, or 11 volumes per cent, in comparison with 41 per cent or 7 6 volumes per cent in the control group Therefore the oxygen tension of the tissues in our cardiac cases must be lower than in normal persons Analysis of arterial and peripheral venous blood has also demonstrated the increased arteriovenous oxygen difference in cardiac disease (21) Lack of oxygen being a

cause of increased permeability (22) this may well be a factor in the production of edema in such cases

Our value for the arteriovenous oxygen difference in the control group is somewhat larger than that obtained by certain other investigators However, our mean value for eight normal young adults, 6 volumes per cent, agrees with that found by Grollman in similar subjects (7) using the acetylene cardiac output method, while that of the whole control group is very similar to the values found by Prodger and Dennig (23) using the same method The smaller arteriovenous differences found in patients by cardiac puncture (24), (25), are probably not comparable with results obtained in the basal state for the patients were doubtless excited by this procedure. The differences between our results and those obtained in experiments in which the oxygen tension of mixed venous blood was calculated from rebreathed gas mixtures presumed to be in equilibrium with it (26), may be due to the fact that the slope of the oxyhemoglobin dissociation curve is so steep at this point, that a slight error would make a large difference in the calculated result

Data bearing on certain clinical conditions

Hypertension The results obtained on the seventeen cases of hypertension deserve special consideration They disclose that the cases with small hearts have a smaller basal cardiac output than those with large hearts In some cases the output per minute is smaller than in many normal persons, as has been demonstrated by others (24, 27) This reduced cardiac output permits the maintenance of hypertension without an increase in the heart's basal work When the basal cardiac work is not elevated, the heart is not enlarged When it is elevated, the amount of enlargement is closely proportional to the increased work. Therefore, our findings are consistent with the well known conception that cardiac hypertrophy is analogous to that which occurs in skeletal muscles after increased work.

The failure of three of our cases of prolonged hypertension (numbers 4, 14, and 16) to develop evidence of cardiac hypertrophy may be attributed to the reduced cardiac output, an adaptation which spares the heart, though perhaps at the expense of other tissues This furnishes a possible explanation for those cases of hypertension who show no cardiac hypertrophy at necropsy, (28) This explanation is consistent with the well known fact that cardiac failure is less likely to occur in cases of hypertension with small than with large hearts

In striking contrast to the close correlation between heart work and heart size in our cases of hypertension, is the complete lack of relationship between cardiac output and body surface area in these cases (Table III, 9 to 12 and 2)

In cases of hypertension the heart conforms to Starling's Law much more closely than is the case in normal individuals This suggests that, when the heart is under strain, the basal cardiac work is determined chiefly by the mass of muscle But when cardiac reserve is unimpaired, the basal cardiac work is more influenced by extracardiac factors

Functional heart disease, hyperthyroidism, anemia, and paroxysmal tachycardia In the other clinical classes the number of cases is too small to permit any generalizations, but certain of the results deserve comment The four cases of functional heart disease are characterized by a low average cardiac output, both absolutely and per square meter of body surface In this they resembled the cardiac group with which they have so many symptoms in common Their arteriovenous oxygen difference, 50 per cent or 9 2 volumes per cent, was midway between the control and cardiac groups The relationship between basal heart work and size was not abnormal because they had smaller hearts than normal

Two of the three cases of hyperthyroidism had a slightly increased cardiac output per square meter of body surface, even though they were taking iodine This feature of hyperthyroidism was first demonstrated by Liljestrand and Stenstrom (29)

The two cases of anemia did not show the increased cardiac output which we expected Both of them appear to have reduced their basal metabolism to a point where a normal cardiac output will carry the necessary oxygen In one of these patients starvation may well have been the cause of this decrease This method of compensating for anemia is not that usually described

The one case of paroxysmal tachycardia showed far less cardiac work per beat during the attack than afterward, a finding similar to that of Barcroft, Bock and Roughton (30) Unfortunately, no orthodiagram could be made during the attack, the patient could not stand In charting the results it has been assumed that the size was unaltered This decrease in work during the attack is perhaps analogous to the decrease of cardiac efficiency which occurs in the heart-lung preparation when the rate is increased (18)

Summary

(1) Duplicate determinations of cardiac output and metabolism, repeated estimations of blood pressure and pulse rate, and orthodiagrams have been assembled in fifty individuals These estimations were performed on fasting subjects lying at rest, after a prolonged rest period Those tested included apparently normal persons, patients who had recovered from congestive failure, and patients with some circulatory abnormality but not immediately threatened with failure, viz thyrotoxicosis, hypertension, anemia, angina pectoris, compensated valvular disease and functional heart disease

(2) When the basal work of the left heart of these subjects is plotted against the volume of the heart or the area of the cardiac silhouette the points representing cases not threatened with failure are found to be arranged about a straight line On the other hand the points representing cases threatened with failure are outside the limits of the normal cases

(3) We regard our results as evidence that Starling s "Law of the Heart" holds for the basal cardiac work in diverse clinical conditions as well as for the heart lung preparation Paraphrasing his words we may say, "Within physiological limits the larger the size of the heart, the greater is the energy of its contraction " And as a corollary, when the work of any heart is not commensurate with its size, that heart is threatened with failure

(4) On the basis of a diverse group of cases believed to have normal myocardia we have made a preliminary estimate of the normal relationship between heart work and size Charts and equations are submitted, by which the question of the normality of any case may be decided

(5) The relationship between heart size and heart work per beat was especially striking in 17 cases of hypertension Those with hearts of normal size, by reducing cardiac output, maintained their hypertension without greater expenditure of cardiac work than normal persons The cases with large hearts were performing increased work. Considering increased cardiac work as cause of hypertrophy in the latter group, its absence will explain the absence of hypertrophy in the former

(6) The cardiac output was directly related to the metabolism in the cases not threatened with failure The arteriovenous oxygen difference was much smaller in these cases than in those who had been decompensated The cardiac output was related to the size of the heart, but, as a rule, not so closely as was the cardiac work. There was a surprising lack of correlation between cardiac output and body surface area in cases of hypertension, the remainder of our control cases showed correlation above the level of significance

(7) Although the errors in estimating basal cardiac output or work are undoubtedly large, the differences found in clinical conditions are so much larger that the results, properly interpreted, have clinical significance.

TABLE IV

Data for 50 cases

Case number	Ethyl iodide				Cardiac output	Pulse rate	Respiration	Blood pressure	O_2 consumed		Heart size	Left ventricular work		Remarks
	Inspired	Expired	Alveolar	Rebreathed										
	mgm per liter × 100	mgm per liter × 100	mgm per liter × 100	mgm per liter × 100	liters per minute	per minute	liters per minute	mm Hg	cc. per minute	per cent basal	sq cm	kgm m per minute	gm. m per beat	
														Class I—Hypertension
1	457	256	163	59	3 5	75	11 2	178/80	255	−1	111	6 8	82	Diabetes mellitus Arteriosclerosis Grangrene of left foot Ethyl iodide distribution coefficient (D C) = 6 9
	417	278	200	83	2 7	75	14 1	164/78	270	+8		4 9	59	
	487	277	149	39	2 0	66	6 5	184/80	240	−7		4 0	55	
	496	273	192	65	2 9	56	10 2	144/68				3 4	55	
	520	318	202	73	1 7	54	6 9	150/66	221	−14		2 8	47	
	548	280	190	72	3 0	54	8 1	170/76	282	+7		5 5	92	
2	583	303	193	57	2 4	56	7 2	150/70	184	−26	126	3 9	62	Same patient as Number 1 6 months later Gangrene healed Angina pectoris D C 6 8
	583	305	189	50	2 4	54	7 2	150/70	209	−20		3 9	66	
	578	323	244	97	3 3	56	12 2	140/100	245	−3		6 0	97	
	580	320	225	64	2 0	54	7 5	140/100	250	−10		3 6	61	
3	515	251	178	42	2 4	59	7 0	148/112	238	+3	109	4 2	72	Diabetes mellitus Gangrene of foot D C 6 0
	528	303	154	23	2 1	55	6 8	148/116	219	−7		3 8	68	
4	517	204	129	48	2 8	70	4 1	195/108	184	−5	105	5 7	82	Known duration 6 years
	514	210	130	34	2 5	72	4 5	200/114	202	+6		5 3	74	
5	546	225	116	31	4 0	96	5 9	200/118	289	+13	134	8 6	90	Symptoms for 1 year
	528	222	125	36	4 0	96	6 5	210/118	287	+12		8 9	93	
6	483	233	117	53	5 0	76	7 2	190/90	227	+10	130	9 5	125	Congenital polycystic kidneys
	507	271	136	50	4 0	76	8 2	194/90	257	+24		7 7	101	
7	621	259	202	89	3 8	76	6 6	166/104	250	+9	129	6 9	92	Lues
	613	294	219	111	4 5	76	8 5	164/100	233	+7		8 1	106	
8	526	275	136	33	2 3	60	5 3	158/98	192	−11	112	4 0	66	Temporary right hemiparesis Known duration of hypertension 4 years
	540	272	143	41	2 4	58	5 1	156/100	186	−13		4 2	72	

TABLE IV (*continued*)

Case number	Ethyl iodide				Cardiac output	Pulse rate	Respiration	Blood pressure	O_2 consumed		Heart size	Left ventricular work		Remarks
	Inspired	Expired	Alveolar	Rebreathed										
	mgm. per liter × 100	mgm. per liter × 100	mgm. per liter × 100	mgm. per liter × 100	liters per minute	per minute	liters per minute	mm. Hg	cc. per minute	per cent basal	sq cm.	kgm. m. per minute	gm. m. per beat	
9	405	173	106	35	3 2	102	5 4	220/120	219	+18	112	7 4	73	Quiet during 1st run Incontinence of urine caused great excitement during 2nd run
	396	190	117	51	5 9	140	10 2	260/152	387	+111		16 5	112	
10	518	244	110	50	5.3	78	6.5	210/130	276	+1	174	12 3	158	Lues Aortic aneurysm
	538	199	120	49	5 6	78	6 7	208/134	291	+6		13 0	167	
11	589	265	151	74	5.3	92	7 2	160/90	251	+48	113	9 0	98	Toxic adenoma of thyroid
	570	237	140	65	5 6	92	7 0	156/90	312	+79		9 4	102	
12	544	238	160	77	6 0	80	11 0	164/78	314	+31	107	9 9	123	Arteriosclerosis Hypertension for 7 years
	537	240	172	64	5 0	72	10.2	160/72	257	+2		7 9	109	
13	535	270	216	122	3 8	80	7.5	148/90	200	+3	95	6 1	77	Lues
	519	315	201	120	3 1	80	6 9	145/95	221	+10		5 1	63	
14	524	255	141	33	2 6	102	5 8	196/104	236	+10	80	5.3	52	Known duration of hypertension 1 year
	532	241	134	33	2.8	100	5 4	206/104	176	−12		5 9	59	
15	592	260	164	68	4 1	72	6 7	182/142	289	+20	149	9 0	125	Malignant hypertension
	594	273	188	90	3 9	72	6 6	182/146	175	−28		8 7	121	
16	604	247	164	40	2 4	50	4 6	148/110	191	−13	109	4 2	84	Duration 4 years at least
	607	261	170	53	2.5	50	4 8	140/118	201	−8		4 4	88	
17	563	261	155	42	2 8	68	5 9	169/88	173	−7	94	4 9	73	Heart normal size. Hypertension of 4 years duration
	560	246	172	42	2.5	72	5.8	170/88	176	−6		4 4	61	
18	566	200	118	45	4 1	60	5 0	170/88	246	−13	150	6 9	116	Apparently normal
	573	212	131	54	4 4	56	4 9					7 5	113	

TABLE IV (*continued*)

Case number	Ethyl Iodide				Cardiac output	Pulse rate	Respiration	Blood pressure	O_2 consumed		Heart size	Left ventricular work		Remarks
	Inspired	Expired	Alveolar	Rebreathed										
	mgm per liter × 100	*mgm per liter × 100*	*mgm. per liter × 100*	*mgm. per liter × 100*	*liters per minute*	*per minute*	*liters per minute*	*mm Hg*	*cc per minute*	*per cent basal*	*sq cm.*	*kgm m per minute*	*gm. m per beat*	
														Class II—Anemias
19	495	173	123	40	3 5	70	4 5	106/64	171	−30	111	4 0	56	Pernicious anemia R B C 2,200,000 Hb 45 D C 5 5
	488	149	109	16	3 0	68	4 1	102/60	165	−33		3 1	45	
20	599	253	158	58	3 8	84	5 2	105/60	208	−10	130	4 2	51	Anemia secondary to gastric hemorrhage Starving R.B C 2,800,000 Hb 33 D C 5 3
	604	265	182	103	4 4	88	4 9	98/60	170	−24		5 0	54	
														Class III—Hyperthyroidism
21	536	213	113	49	5 0	112	5 6	120/60	272	+43	73	6 1	55	Diffuse toxic goitre
	527	215	117	49	4 4	108	5 3	114/60	248	+27		5 2	48	
22	497	242	145	34	3 4	96	8 2	138/64	331	+62	116	4 7	48	Diffuse toxic goitre Under iodides
	497	248	162	33	2 9	95	8 5	152/66	356	+79		4 3	45	
23	574	233	177	61	4 3	128	8 2	120/58	377	+87	127	5 2	41	Diffuse toxic goitre Under iodides
	567	234	178	86	5 4	140	8 3	116/55	302	+58		7 3	52	
														Class IV—Threatened decompensation
24	582	241	169	66	3 4	92	5 7	125/30	262	+22	158	3 6	39	Rheumatic heart disease Mitral stenosis, mitral and aortic regurgitation, under digitalis
	583	243	168	76	3 8	92	5 8	122/30	246	+12		3 9	43	

TABLE IV (*continued*)

Case number	Ethyl iodide				Cardiac output	Pulse rate	Respiration	Blood pressure	O_2 consumed		Heart size	Left ventricular work		Remarks
	Inspired	Expired	Alveolar	Rebreathed										
	mgm. per liter × 100	mgm. per liter × 100	mgm. per liter × 100	mgm. per liter × 100	liters per minute	per minute	liters per minute	mm. Hg	cc per minute	per cent basal	sq cm.	kgm. m per minute	gm. m per beat	
25	513	343	210	61	1 8	72	10 4	230/130	157	−21	145	4 4	61	Hypertension Angina pectoris Marked discomfort during 1st run 2nd run satisfactory No digitalis
	512	343	227	41	1 1	72	7 0	224/128	136	−35		2 7	37	
26	607	367	273	98	1 9	102	7 8	118/76	241	+3	184	2.5	25	Rheumatic heart disease. Advanced mitral stenosis. No digitalis
	611	377	281	122	1 9	92	7 4	110/75	230	−2		2 4	26	
27	526	281	201	85	3 7	96	10 1	182/124	310	+35	200	7 9	82	Myocardial degeneration Cause unknown No digitalis
	525	283	209	97	3.3	92	8 6	180/126	273	+18		7 0	76	
28	613	313	188	63	2 6	120	6 0	130/85	244	+34	127	3 4	29	Toxic goitre Auricular fibrillation Under digitalis
	629	320	198	68	2 5	120	6 0	140/85	239	+30		3 2	27	
29	573	322	201	48	2 5	112	8 6	118/100	333	+34	154	3 7	33	Myocardial degeneration Obesity Under digitalis
	573	317	198	56	2 9	104	9 0	122/102	310	+25		4.3	41	
														Class V—Angina pectoris
30	507	322	223	73	3 2	76	14 6	112/70	269	+18	96	4 0	52	Alv CO_2 2 per cent at end of run
	504	337	224	65	2 6	74	13 8	116/70	287	+23		3.3	45	
31	565	310	153	42	2 0	97	4 9	110/65	188	−3	81	2 4	25	Coronary occlusion 3 years before. Duplicate lost because of pain Died 2 weeks later

TABLE IV (*continued*)

Case number	Ethyl Iodide				Cardiac output	Pulse rate	Respiration	Blood pressure	O_2 consumed		Heart size	Left ventricular work		Remarks
	Inspired	Expired	Alveolar	Rebreathed										
	mgm per liter × 100	*mgm per liter × 100*	*mgm per liter × 100*	*mgm per liter × 100*	*liters per minute*	*per minute*	*liters per minute*	*mm Hg*	*cc per minute*	*per cent basal*	*sq cm.*	*kgm m per minute*	*gm m per beat*	
														Class VI—Functional heart disease
32	573	139	82	24	7 5	113	5 6	126/80	269	+16	75	10 5	93	Neurocirculatory asthenia
	552	156	89	34	6 8	110	5 3	128/80	246	+6		9 5	87	
33	492	284	174	48	2 2	88	7 4	120/80	231	+2	92	3 0	34	N C A
	497	305	189	61	1 9	84	7 1	110/70	265	+17		2 3	28	
34	523	201	112	47	3 3	74	3 6	104/70	205	−17	77	3 9	53	N C A
	530	182	115	59	4 2	78	3 8	108/70	211	−15		5 1	65	
35	562	302	187	44	1 3	72	4 1	98/60			69	1 4	19	N C A.
	571	327	176	64	1 9	68	4 9	100/62	174	−6		2 1	31	
36	598	270	157	51	2 6	88	4 6	103/68	219	−13	79	3 0	34	N C A
	594	272	155	66	3 1	88	4 8	100/65	217	−11		3 5	40	
														Class VII—Normal circulation
37	536	229	132	34	2 6	80	4 6	96/50	193	−5	75	2 6	32	Normal
	535	222	131	52	3 5	78	4 9	94/50	203	−2	77	3 4	44	
38	534	314	175	56	1 7	72	5 1	110/74	189	−19	85	2 1	30	Gastric neurosis
	527	318	172	68	1 9	68	5 3	106/72	182	−21		2 3	34	
39	504	232	113	34	3 0	66	4 9	98/58	210	−15	83	3 2	48	Visceroptosis
	506	244	119	26	2 4	68	4 8	98/60	201	−19		2 6	38	
40	496	249	231	127	4 0	60	9 4	116/78	210	−16	88	5 3	88	Psychoneurosis
	507	241	190	100	3 4	60	6 4	110/70	221	−17		4 2	69	
41	571	295	160	39	2 1	46	5 2	98/60	187	−21	99	2 3	49	Diabetes mellitus
	578	303	159	46	2 3	46	5 2	99/60	202	−14		2 5	54	

TABLE IV (*continued*)

Case number	Ethyl iodide				Cardiac output	Pulse rate	Respiration	Blood pressure	O_2 consumed		Heart size	Left ventricular work		Remarks
	Inspired	Expired	Alveolar	Rebreathed										
	mgm. per liter × 100	mgm. per liter × 100	mgm. per liter × 100	mgm. per liter × 100	liters per minute	per minute	liters per minute	mm. Hg	cc. per minute	per cent. basal	sq. cm.	kgm. m. per minute	gm. m. per beat	
42	586	302	158	44	2.5	62	5.7	110/70	202	−15	114	3.1	50	Chronic cholecystitis (?)
	596	262	168	37	2.7	62	6.0	110/70	213	−11		3.3	53	
43	508	170	95	32	4.9	64	5.1	120/70				6.3	99	Normal
	498	185	103	48	5.3	66	5.2	120/70	262	−7	106	6.8	104	
44	522	231	137	49	3.1	60	5.3	115/75	217	−20	117	4.0	67	Normal
	528	252	141	57	3.1	66	5.3					4.0	61	
45	564	184	120	47	4.3	50	4.6	120/80	237	−14	112	5.8	117	Normal
	558	191	128	61	4.2	46	4.4					5.7	124	
46	510	203	103	41	3.5	72	3.9	120/80	224	−17	87	4.8	66	Normal
	504	197	122	52	3.2	68	4.1					4.3	64	
47	596	310	201	85	4.0	72	9.7	120/65	269	+17	108	5.0	70	Normal
	578	295	210	95	4.1	68	9.5					5.1	76	
48	576	260	124	44	3.6	58	5.1	120/78	253	+2	142	4.8	84	Normal
	574	252	140	55	3.6	54	5.4					4.8	90	
49	576	217	121	51	5.2	64	5.7	115/68	250	−4.5	114	6.6	104	Rheumatic fever in childhood with cardiac involvement
	573	228	128	64	5.5	68	5.8		255	−2		7.0	103	Slight, if any, evidence of valvular disease at present
														Class VIII—Paroxysmal tachycardia
50	557	335	230	67	1.7	142	6.9	106/86	237	+5		2.2	16	Advanced rheumatic heart disease during paroxysm
	519	333	244	78	1.5	132	6.6	102/82	231	+3		1.9	14	
50	598	256	153	45	2.3	56	4.2	106/74	154	−30	123	2.9	52	Same patient day after paroxysm
	592	266	164	51	2.3	60	4.6	108/76	176	−21		2.9	48	

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THE TREATMENT OF AGRANULOCYTOSIS WITH ADENINE SULPHATE[1] [2]

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On December 20, 1928, a young physician suffering from agranulocytosis was given the salt of a purine base, adenine sulphate, intravenously and recovered promptly (1) This, as far as can be ascertained, was the first time such a substance had been tried for this condition Its use was prompted by the fact that previous animal experimentation suggested that purine salts, and some of the substances from which purines are derived, seemed effective in increasing granulocytes in the peripheral blood stream (2) (3) (4) Since the first report (1) of its use, adenine sulphate has been given by us, or its administration reported to us in 35 cases

Of these patients, 15 were definitely diagnosed as having had "primary" agranulocytosis, showing a marked diminution of granulocytes with or without inflammation of the pharynx, 8 showed agranulocytosis, either characterized by serious complications or occuring in the course of some other illness and 12 individuals had some definite condition other than agranulocytosis and the depression of granulocytes was either incidental or occurred as part of the general picture

Most of these patients were treated rather cautiously with what was at first considered adequate dosage With more experience, especially with respect to the relative non toxicity of the adenine salt, we now use as the present dosage for an adult 1 gram boiled in 35 or 40 cc of physiological saline, administered intravenously, sufficiently warm to prevent precipitation, 3 times a day for at least three days Even this is probably not a maximum dose, but in most patients who responded favorably it was found that with such quantities distinct improvement occurred in symptoms, in decline of fever and in increase of granulocytes within 48 hours, frequently within 24 hours As much as 10 4 grams have been given to a patient within 7 days with no ill effects It is necessary to emphasize this question of dosage to arrive at a proper evaluation of the results to be reported

[1] Acknowledgment is made to the physicians whose names appear in the tables, and who permitted us to use the results which are given in this study

[2] Adenine sulphate has been obtained from the Eastman Kodak Company of Rochester, New York

Table I summarizes the results obtained with a group of 15 patients who suffered from what might be termed agranulocytosis "vera," because they showed the classical signs and symptoms of this condition

The results obtained in treating this group give a total recovery rate of 73 per cent which is about the same as that obtained by Jackson (5) with the more complex nucleotide A more detailed analysis of these cases shows that of the four patients who did not recover, three died within 15 hours after the administration of adenine sulphate was begun, and the fourth within two days This last patient was given a transfusion and, what is even more significant with respect to a depressing effect on the bone marrow, neosalvarsan intravenously One of these fatal cases received a transfusion of 900 cc of blood and salvarsan In two of these fatal cases only 1 dose of 0 5 gram of adenine sulphate was given, in one, 2 doses of 0 5 gram, and in one, 3 doses of 0 8 gram It should also be pointed out that three of the patients who recovered showed improvement as far as appearance of granulocytes was concerned, before adenine sulphate was administered This is a very significant observation because in evaluating adenine sulphate therapy it is essential to keep constantly in mind the possibility of spontaneous recovery Of the patients who recovered, almost all showed evidence of response to adenine sulphate within 24 hours after the administration of the purine salt, as evidenced by the beginning increase in granulocytes, drop of temperature and symptomatic improvement, and most of the patients demonstrated very distinct improvement within 48 hours This quick reaction is important, of course, because of the acuteness of the disease It is not the purpose of this paper to analyze the individual cases, except as is necessary to follow the therapy, but attention should be called to the low initial total and polymorphonuclear counts of most of the recovered patients

The second group of 8 cases (Table II), includes patients in whom the diagnosis of agranulocytosis was questionable, or in whom what might have been either the primary disease or serious complications of agranulocytosis dominated the picture The disease did not represent a clear cut clinical entity, and all that can be definitely stated is that a blood picture of agranulocytosis was present The possibility must, of course, be considered that this group of cases represents a late stage of the typical agranulocytic condition from which the patients have not recovered quickly, either as a result of therapy or spontaneously Only one patient of this type recovered after adenine sulphate therapy In this group, therefore, the recovery rate was one out of eight An analysis of Table II shows that 3 patients had marked anemia, two had marked lymphadenitis, one suffered from multiple bone marrow abscesses of pneumococcus origin and septicemia, two had cardiac failure, one had pulmonary edema, in one there was a question as to whether the diagnosis

Case	Initial count		Final count		Number of injections of adenine sulphate	Total quantity injected	Duration of time between 1st injection and distinct recovery	Duration of time between 1st injection and death	Remarks. Complications
	Leukocytes	Polymorphonuclear	Leukocytes	Polymorphonuclear					
	thousands	per cent	thousands	per cent		grams			
Dr Russel's case, M H ♀	1.8	0	1 1	2	1	0.5		15 hours	
Dr Parson's case 1, J B ♀	0 8	0	10 1	57	10	5 0	4 days		1 x ray dose. Transfusions
Dr Kobocker's case ♀	1 4	20							
	1 6	4	6 0	35	several*	?	?		Beginning spontaneous recovery before adenine was given
	4 0	20							
Dr Pincus case Mrs N ♀	1 3	0	6 4	59	2	1 0	2 days		Relapse, and after 2d injection, reaction Now on nucleotide
	2 0	24							
Dr Gibbs and Dr Steinberg's case C B ♂	3.2	6	12 4	70	7	3.5	2 days		Trench mouth 4 weeks before Salvarsan 2 weeks before
Dr Reid's and Dr Belcher's case K N ♀	1 4	10	9 0	54	13	10 4	2 days		Left tonsil sloughed out
Dr Disbrow's case, M L P ♀	0 5	0	0 2	0	2	1 0		2 days	Neosalvarsan intravenously 280 cc. transfusion before remedy
Dr Dimeshek's case R S ♀	0 8	0	21 0	?	2	1 0	12 hours		Type IV pneumococcus
Dr Francis case R B ♂	7 8	3	15.3	70	4	1 1	2 days		3 years old
Dr Eggston's case 1 Miss C ♀	2 0	0	1 1	0	1	0 5		4 hours	Salvarsan Blood transfusion 900 cc
	1 4	0							
Dr Eggston's case 2 ♂	0 7	0			3	2 4		14 hours	*
Dr Burcky's case ♀	1 0	1	6 2	62	4	2 0	36 hours		
Dr Murphy's case M V ♀	0 7	4	7 4	64	1	0 5	3 days		Transfusion Recovery attributed by physician to some other drug
Dr Linn's case ♀	1 0	0	3.5	22	6	3 0	Within 12 hours		
				40					
Dr Lange's case, F M ♀	1 0	8	2 8	40	15	7 5	3 days		
			2 0	20					

* Before adenine sulphate was given, patient had transfusions, x ray, intramuscular whole blood, leukocyte extract with no response One week after recovery patient developed laryngeal obstruction had tracheotomy developed leukocytosis and polynucleosis and had fatal hemorrhage at site of tracheotomy Count before suffocation, white blood cells 43,000, 88 per cent polymorphonuclears

TABLE II

Agranulocytosis—complicated or questionable

Case	Initial count		Final count		Number of injections of adenine sulphate	Total quantity injected	Duration of time between 1st injection and distinct recovery	Duration of time between 1st injection and death	Remarks Complications
	Leukocytes	Polymorphonuclears	Leukocytes	Polymorphonuclears					
	thousands	*per cent*	*thousands*	*per cent*		*grams*			
Dr Russel's case, W K ♀	2 9	2	9 3	80	2	2 0	10 hours distinct 1 day marked		Lymphadenitis, rheumatic heart, "secondary anemia," angina
Dr Pease's case, M L ♀	0 72	0	0 7	3					Red blood cells 1,400,000, hemoglobin 30 per cent Septicemia, multiple bone marrow abscesses, pneumococcus 3 years old Transfusions 350 and 150 cc
	1 0	0	1 1	1	4	1 1		3 days	
			0 4	0					
Dr Gibb's case, M S ♀	0 44	1	1 1	3	2	1 4		3 days	2 transfusions, 500 cc Nucleotide, 5 and 10 cc Edema of lungs, acute fibrinous pleuritis
Dr Grimmer's case ♂								?	Cardiac decompensation X-ray therapy
Miss S ♀	0 4	0			1	0 5		Within 18 hours	Cervical adenitis, acidosis
Dr Goodman's case ♂								Within 24 hours	Leukemia? Agranulocytosis?
Dr Manheim's case, H H ♀	1 3	20	4 3	60	19	8 5		2 months	Abscess of jaw—necrosis Neosalvarsan Transfusions 13—6,500 cc
			1 2	8	At 2–7 day intervals, 0 5 gram doses				
Dr Pulsifer's case ♂	2 6	0	0 4	0	4	2 0		2 days	*

* Citrated blood transfusions, 500 cc twice, 400 cc once Postmortem showed cellulitis of neck, necrotizing bronchopneumonia, acute esophagitis, acute splenic tumor, secondary anemia, pulmonary edema, chronic cholecystitis, cholelithiasis, advanced arteriosclerosis

was not really aleukemic leukemia, one had necrosis of the jaw, and one patient showed cellulitis of the neck, necrotizing bronchopneumonia, and chronic cholecystitis and cholelithiasis In addition to adenine sulphate 4 of these patients received transfusions, one, x ray therapy, and one nucleotide. Of the patients who died only one received more than 2 0 grams of adenine sulphate This patient (H H) had a total of 8 5 grams given to her over a period of two months, and at one time her count rose to 4,300 leukocytes with 60 per cent polymorphonuclear cells

Table III presents the results obtained with adenine sulphate in 12 patients who suffered from some disease other than agranulocytosis but in whom agranulocytosis developed during the course of illness or was a concomitant finding Of these, 3 patients suffered from aleukemic leukemia, 5 from lues and anti luetic (arsenicals) therapy, 1 from a mastoid infection, 1 from carcinoma of the prostate, 1 from generalized abdominal carcinomatosis, for which she had received radiation therapy, and 1 from staphylococcus septicemia Aleukemic leukemia was included in this group for the following three reasons (*a*) because of a report of a possibly successful outcome with nucleotide (6), (*b*) because in some of these cases agranulocytosis was diagnosed and treatment commenced before the true condition was apparent and (*c*) because in a strict sense this condition is certainly accompanied by some degree of granulocytopenia The cases of arsphenamine poisoning really should be classified as aplastic anemia or probably acute myelophthisis since all blood elements are involved Here again agranulocytosis is part of the picture This problem of classification has been thoroughly discussed by Rosenthal (7) and by Roberts and Kracke (8) In this series the recovery rate with adenine sulphate therapy was one out of twelve The patient who recovered was a child who subsequently had a relapse after his operation for mastoiditis After a single dose of 0 3 gram of adenine sulphate and transfusions, the child's count rose from 1,400 leukocytes and 6 per cent polymorphonuclears to 6,900 leukocytes and 60 per cent polymorphonuclears Adenine sulphate was not administered during the relapse, and he was treated only by transfusions After the relapse the patient s count varied from 1,200 leukocytes to 1,000 leukocytes and showed 0 per cent polymorphonuclears despite frequent transfusions The patient suffering from staphylococcus septicemia had a rise of leukocytes from 2 000 to 29,600 and of polymorphonuclears from 2 to 94 per cent in 6 days but died 4 days later The patients suffering from aplastic anemia following arsphenamine therapy and those suffering from aleukemic leukemia showed no change in blood count following the administration of adenine sulphate.

TABLE III

Agranulocytosis complicating other conditions

Case	Initial count		Final count		Number of injections of adenine sulphate	Total quantity injected	Duration of time between 1st injection and distinct recovery	Duration of time between 1st injection and death	Remarks Complications
	Leukocytes	Polymorphonuclears	Leukocytes	Polymorphonuclears					
	thousands	*per cent*	*thousands*	*per cent*		*grams*			
Dr Eggston's case 3, child ♂	0 9	0	adenine		1	0 3	Few days?	Several weeks	Mastoid—relapse later to death No adenine given during relapse Several transfusions
			1 4	6					
			transfusion						
			6 9	60					
			operation						
			1 2	0					
			1 0	0					
Dr Clark's case, J S ♂								Within 24 hours	Leukemia?
R C ♀	2 9	9	2 3	29	5	4 8		27 days	Alveolar abscess Probably a type of aleukemic leukemia, because of great numbers of blasts in blood smear
Dr O'Regan's case ♂	4 6	12	6 1	30	7	4 9	Still alive		Smear now contains blasts and patient is probably suffering from leukemia
Dr Miller's case ♂								1 year	"No effect" Carcinoma of prostate
Dr McPherson's case ♀	0 2	0			1	0 5		Within 6 hours	Carcinomatosis (abdominal) Radiation therapy
Dr Keasbey's case 1 ♂	2 0	2	29 6	94	6	3 0		10 days	Staphylococcus septicemia
			In 6 days						
Dr Parson's case 1, H F ♂	0 7	24	0 2	26	3	1 5		2 days	Moribund when admitted Aplastic anemia Salvarsan therapy Transfusion
			0 4	36					
Dr Parker's case ♀								?	Temporary rise in polymorphonuclears Aplastic anemia, neosalvarsan poisoning
Dr Keasbey's case 2 ♂	2 4		0 25		18	9 0		2 weeks	Antiluetic panmyelophthisis
Dr Carter's case, M W ♀	1 3	10			1	0 5		6 hours	Syphilis, salvarsan therapy, adenitis, posttonsillar abscess Hydrothorax, acute peritonitis, ulceration of colon Mercury poisoning Transfusion
Dr Carter's case, M P ♂	0 8	4	2 4	8	10	8 0		7 days	Syphilis, arsphenamine therapy, adenitis

DISCUSSION

The subject of agranulocytosis has been reviewed so often recently that it would be superfluous in this limited study on therapy to discuss the condition in detail It should be pointed out that probably the first case to be described was that by an American, Brown (9) in 1902 Schwarz in 1904 (10) and Türk (11) in 1907 published case reports, and in 1922 Schultz (12) first described the syndrome of agranulocytosis in some detail However, there is justifiable doubt concerning the advisability of classifying this condition as a clinical entity Hartwich (13) has summarized the evidence recently and his conclusion that agranulocytosis is a symptom complex which mirrors a constitutional inferiority of the bone marrow seems to be the most acceptable viewpoint at present

Aside from the symptomatic treatment, the chief methods of therapy tried in any considerable number of cases of agranulocytosis are, (*a*) radiation, (*b*) transfusions and (*c*) purine or nucleotide therapy These latter chemicals are grouped together because in all probability the active principle in them is the same The chief exponents of radiation therapy are Friedemann and Elkeles (14) Taussig and Schnoebelen (15) and Gager and Speer (16) also reported a small number of cases In 1928 Friedemann (17) reported that 6 of 10 patients, 60 per cent, treated by x ray recovered In 1930, Friedemann and Elkeles (14) reported a total of 43 cases with recovery of 13, 30 per cent. However, they point out that 23 of these patients had sepsis or pneumonia Therefore, x ray therapy has been followed, in uncomplicated cases, by a recovery rate of 65 per cent. If the further deduction of 5 is made for the patients who died within 36 hours after onset of treatment, the recovery rate with radiation is raised to 87 per cent Even if such deductions are permissible, the chief cautions to be observed, in evaluating this method of therapy, are the time element that must elapse in these very acute cases before the supposed effect of radiation is manifested, and the absence of proof that radiation actually stimulates granulocytopoiesis If radiation stimulates the granulocytic elements of the bone marrow by contiguous hyperemia or fixed tissue cell proliferation, subsequent atrophy of the granulocytes may result That this is not entirely a theoretical objection, is suggested by a study of two of the cases reported by Taussig and Schnoebelen (15) The patients had an acute relapse after recovery from an attack in which radiation was used as a therapeutic measure Radiation was completely ineffective in the relapse

Transfusions are given constantly in agranulocytosis although no extensive series of cases treated by this method, has been reported As far as is known, transfusions or intramuscular injections of blood have never been shown to stimulate the production of polymorphonuclear leukocytes Hartwich (13) advocates its use solely for its effect temperature Our experience suggests that a large trans-

actually depress the bone marrow (1) Small transfusions may be given but there is certainly no evidence that this method of therapy is of specific value

The use of adenine sulphate and nucleotide has been shown to be effective in more than 70 per cent of uncomplicated cases of agranulocytosis (5) (18) Although adenine sulphate has the disadvantage of being less soluble than nucleotide, it has been given at least 150 times with only two reports of minor constitutional reactions Moreover, it is a simpler chemical product than nucleotide, and when effective, it produces some improvement in 24 hours and a distinct return towards normal in 48 hours Salts of adenine, which are much more soluble, such as the lactate, the acetate, and the chloracetate of adenine, have been given to rabbits, and found to be non-toxic when administered either intravenously or intramuscularly The clinical study of these more soluble adenine salts in agranulocytosis will soon be undertaken

It must be emphasized that in a disease such as agranulocytosis spontaneous recovery is so common that any evidence such as has been reported in this study may be only circumstantial Production of experimental agranulocytosis, more nearly simulating the disease than has been done in the past (19) (20), is essential before more definite proof of the value of therapeutic agents can be established

CONCLUSIONS

1 Adenine sulphate therapy in 15 uncomplicated cases of agranulocytosis has been followed by recovery in 11 of the patients

2 In severely complicated cases of agranulocytosis or in aleukemic leukemia and aplastic anemia, adenine sulphate has not been effective in the doses used in this study

3 One gram of adenine sulphate boiled in 35 to 40 cc of saline, given warm, intravenously three times a day, for at least three days for an adult, is nontoxic, and is suggested as the dose in treating agranulocytosis in adults

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STUDIES IN SO-CALLED WATER INTOXICATION

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INTRODUCTION

Water retention and hydremia have attracted considerable attention in the last decade In 1921 Miller and Williams (1) reported observations on three patients with hypertension and nephritis who were given 5 to 10 liters of water in 24 hours by Rehfuss tube. Two of these patients developed headache, dizziness general depression, increased blood pressure and cramps in the legs The third patient developed no such symptoms presumably because of extremely rapid elimination of water by the kidneys

The following year (1922) Weir, Larson and Rowntree (2) reported observations on patients with diabetes insipidus In these patients urination had been suppressed with pituitary extract while they continued to take their customary large amounts of fluid Headache nausea and vomiting resulted Further observations were carried out on animals Asthenia, salivation, vomiting, tremor, muscular twitching ataxia, convulsions, coma and death occurred when large amounts of distilled water were given by stomach tube, both with and without pituitary injections Dilution of the blood could not be demonstrated in either the patients with diabetes insipidus or in the experimental animals The total nitrogen and chloride content of the plasma usually decreased slightly after the onset of symptoms These investigators failed to find an explanation of the symptoms in edema of the brain, increase in blood volume or significant blood pressure change

Rowntree (3) continued the investigation and in 1923 reported that the syndrome of water intoxication had been produced in dogs, cats rabbits and guinea pigs He found that intravenous salt solution prevented as well as cured the syndrome which could not be produced by rectal administration of water or by an equal amount of normal saline given orally An increased intracranial pressure was demonstrated in one animal at the time symptoms developed and a subsequent autopsy showed what was considered to be ' increase of fluid within the brain substance Rowntree concluded that ' water intoxication is accompanied by and is probably due to, increased intracranial pressure this in turn is probably a manifestation of disturbance in the water salt equilibrium of the central nervous system '

About the same time Moss (4) in England described the loss of large amounts of chloride in colliers by sweating The miner's cramp which sometimes developed in these men he attributed to this loss and the coincident excessive water intake He was able to prevent cramp and fatigue associated with it by the administration of chloride

[1] With the technical assistance of Etta Beer

The same year Weir (5) reported that the patients with diabetes insipidus he had studied *did* have a dilution of the blood which accounted for the slight reduction of plasma chloride

In 1924 Greene and Rowntree (6) reported that they had also found in their experimental animals a definite dilution of blood as shown by reduction of serum protein, increase in plasma volume, and decrease in hemoglobin, but that the decrease in blood chloride often exceeded that expected from the degree of dilution The possibility that this was due to loss of chloride by diuresis was ruled out as the decrease in blood chloride was quite as marked in the animals with oliguria or anuria as in those with the most marked diuresis

Contrary findings were reported by Underhill and Sallick (7), who, while agreeing that the blood became diluted and that the magnitude of the blood chloride reduction exceeded the degree of hemodilution, claimed that a large amount of chloride was lost in the urine They concluded that "the loss of tissue salts with consequent disturbance of the water-salt equilibrium of the body constitutes an important factor in the mechanism of water intoxication"

In 1927 Greene and Rowntree (8) confirmed their previous reports and added that "a trace of chlorides is present in the vomitus but this is inconsiderable by comparison with the changes in the body as a whole"

Misawa (9) using rabbits and the same experimental procedure as Rowntree demonstrated that in water intoxication there was a reduction of chloride in all the tissues of the body save the liver, as well as in the blood The reduction was greatest in skeletal muscle where it amounted to a loss of 51 per cent and it averaged 20 per cent in other tissues, except in liver where a 10 per cent increase occurred There was an increase in the water content of all tissues examined This amounted to 2 per cent in brain, 4 per cent in muscle, and was highest, 5 per cent, in the liver Misawa claimed that the concentration of chloride in the urine increased with the progress of the intoxication Hemolysis and death appeared if water administration was continued after convulsions appeared He considered that the symptoms of water intoxication were not due chiefly to water retention in the tissues or hydremia, but were much more dependent on the marked reduction of inorganic salts in the tissues and lowering of the molecular concentration of the serum

Harding and Harris (10) have more recently repeated the experiments of Rowntree, particularly testing the effect of intravenous hypertonic urea solution, which they did not find of definite therapeutic value They believe that the convulsive seizures of water intoxication occur when the retention of water has reached a critical value of 60 to 70 cc per kilogram

Clinically, McQuarrie (11) and Fay (12) have shown that epileptic convulsions are aggravated by the retention of water McQuarrie (13) emphasizes particularly the changes in mineral balance which occur during the period of water retention in epileptics

EXPERIMENTAL PROCEDURES

Our own experiments were started in August 1930 Dogs, which had not been fed after 11 00 A M of the previous day, were used Distilled water was administered by stomach tube at half hour intervals, in amounts varying from 20 to 75 cc per kilogram of body weight. In the first experiments, however, 50 cc per kilogram were used, as in Rowntree's procedure An initial blood specimen was taken before the administration of water was started and a final specimen at the end of the experiment. Water administration was continued until definite toxic symptoms, and, in many cases, convulsions occurred

Occasionally this required only 3 to 4 hours, but with larger animals a 7 or 8 hour period usually elapsed before the convulsive state was reached

The blood was taken from the external jugular vein Part of the sample was discharged under oil with oxalate while with a second syringe blood was obtained without an anticoagulant for the sedimentation tube and fragility test, and serum for total base, protein and calcium Hemoglobin and hematocrit measurements were made with blood obtained directly from the needle

In the earlier experiments the urine and vomitus were collected and measured together as had been done by previous workers In the later experiments these excreta were collected and analyzed separately

In addition to the chemical studies data on sedimentation, hemoglobin and red blood cell count and size have been collected Fragility tests were also made in many experiments At this time only observations on the chemical changes and their interpretation are presented

The chemical methods used were the following blood chloride, Van Slyke (14) blood sugar, Folin and Wu (15) nonprotein nitrogen, Folin and Wu (16) CO_2 content, Van Slyke gasometric (17), pH, Hastings and Sendroy bicolorimetric (18) inorganic phosphorus, Fiske and Subbarow (19) inorganic sulfates Wakefield (20) urea, Leiboff (21) lactic acid, Friedemann (22) serum albumin and globulin, Greenberg s modification of Wu's method (23) total base, Stadie and Ross (24) urinary and gastric chlorides, Harvey (25)

EXPERIMENTAL RESULTS

The dogs, for the most part, mongrel or street types, varied considerably in clinical response. Some high strung apprehensive animals (e g fox terriers) developed convulsions more readily than more staid types In general, the younger and smaller dogs were more unstable It was found that the animals which vomited early and frequently became toxic sooner, exhibiting at first tremors and ataxia, then prostration and convulsions The effect of intravenous salt solution in allaying these symptoms was, in most instances, spectacular and practically immediate In some experiments intravenous urea was tried but was without effect even when given for several days previous to the experiment as well as at the appearance of convulsions The hemoglobin and hematocrit show more dilution of the blood after 20 to 26 cc of water per kilogram every half hour (experiments 2 and 3, Table I) than after 75 cc. per kilogram every half hour (experiments 4 and 5, Table I) Even in the same animal and with the same dosage of water (experiments 4 and 5, Table I) the hemodilution is not the same. Although the blood dilution was greater, clinical manifestations were less marked and convulsions were provoked with more difficulty with the *smaller* amount of water Weight increase which has been stressed by Harding and Harris (10), is not a reliable index of the amount of water absorbed by the blood or body tissues. In experiment 2 (Table I) the weight change indicates a marked water retention, and hemoglobin and hematocrit a hemodilution, yet there is little clinical evidence of intoxication In experiment 5 (Table I) with a marked increase in weight and little or no hemodilution, convulsions occurred.

TABLE I

Analysis of experiments

Dog	Experiment number		Weight	Chlorides			Plasma CO_2	Hemoglobin (Sahli)	Blood cell volume (hematocrit)	Red blood cells	Plasma pH	Blood sugar	Water given			Total water lost	Chloride loss			Remarks
				Whole blood	Plasma	Cell							Duration	Rate	Total	Urine and vomitus	Urine	Vomitus	Total	
			kgm.	*mgm per 100 cc.*	*mgm per 100 cc.*	*mgm. per 100 cc.*	*volumes per cent*	*per cent*	*per cent*	*millions per cu. mm*		*mgm. per 100 cc.*	*hours*	*cc. per kgm per ½ hour*	*cc.*	*cc.*	*grams of NaCl*	*grams of NaCl*	*grams of NaCl*	
N	2	A	18.0	470	506	347	62 0	85	47			76	7½	20	5 325	2 560		0		No vomiting in this experiment. Occasional shivering and slight twitching toward end only
		B	20 1	456	555	294		74	38			130								
M	3	A	20.5	484	498	450	47.2	56	29			98	7½	26	8,250	4,820				Drowsy Ataxia. Twitching. Slight subcutaneous edema present.
		B	21 8	424	484	253	51 0	52	26			107								
M	4	A	20 0	521	609	213	57.3	65	33			91	7½	75	22 500	19 135	1 0	12 4	13 4	Twitching spasmodically Shallow slow breathing. Very sick, preconvulsive.
		B	22.0	323	387	201	78.7	61	30			130								
M	5	A	19 0	464	597	130*	48.6	70	29	4.60		78	8	75	23,800	20,000	0 0	0 4	10.3	Convulsion
		B	21 6	342	415	150	57.2	71	28	5 05		154								
N	6	A	16 8	547	680	419	54 0	91	51	5.25		80	9	75	22,680	19 795	1 1	6 0	8.0	Quiet. Shivering.
		B	18.7	359	388		65 0	101		5 75		129								
R	7	A	8.7	465	572		57 8	78			7 40	101	7	50	5,850	4 710	0.1	2.7	2.8	Convulsion.
		B	9.2	356	429		68.1	73			7 40	204								
P	8	A	7 0	462	579		47.8	73			7 40	73	4	50	3 000	2,560†	0 1	1.5	1.6	Convulsion with bleeding from mouth.
		B	7 4	380	485		58.6	66			7 40	234								
R	9	A	8.5	467	580		55 7				7 40	92	6½	50	5,100	4 235	0.2	2.2	2.4	Salivating and twitching.
		B	8.8	355	468		62 4				7 45	123								
S	10	A	5 7	459	597	185	47 8	62	33	4.25	7 42		5	50	2,565	1 850	0 0	1 0	1 0	Convulsion No urination during this experiment.
		B	6.0	322	394	161	52.0	54	31	3 90	7 45									

TABLE I (*continued*)

Dog	Experiment number		Weight	Chlorides			Plasma CO_2	Hemoglobin (Sahli)	Blood cell volume (hematocrit)	Red blood cells	Plasma pH	Blood sugar	Water given			Total water lost	Chloride loss			Remarks
				Whole blood	Plasma	Cell							Duration	Rate	Total	Urine and vomitus	Urine	Vomitus	Total	
			Kg.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc	volumes per cent	per cent	per cent	millions per cu. mm.		mgm. per 100 cc.	hours	cc. per kgm. per ½ hour	cc.	cc.	grams of NaCl	grams of NaCl	grams of NaCl	
T	11	A	13.1	482	438	390	50.4	69	36	5.68	7.45	99	4½	50	6 600	5,580	0.4†	3.7‡	4.1	Preconvulsive. Died following giving concentrated Ringer's (25 per cent)
		B		344	451	204	48.3	65	34	5.10	7.55	129								
V	12	A	7.5	470	567	313	49.0	71	38	6.25	7.45	93	8	50	6,000	5,055	0.5	3.2	3.7	Convulsion.
		B	8	3.5	451	199	47.0	66	33	4.93	7.50	137								
T	13	A	13.1	489	610	225	49.0	85	43	5.53	7.45	91	2½	50	4 600	3,033	0.1	3.1	3.2	Convulsion.
		B	14.4	379	458	...8	47.1	77	40	5.50	7.55	151								
N	14	A	1.9	448	582	349	60.7	88	58				8	50	14,400	11,615	0.6	6.1	6.7	Not sick. Quiet.
		B	20.0	383	432	312	68.1	88	58											
R	15	A	8.2	477	596		57.2	85			7.40	80	7	50	8,850	4,635	0.2	2.7	2.9	Convulsion.
		B	8.5	3.5	430		47.3	76			7.40	178								
S	16	A	6.2	466	605	290	41.9	71	42	6.50	7.55	83	3½	50	3,540	2 140	0.2	1.9	2.1	Convulsion.
		B	6.6	323	434	123	31.7	69	38	6.05	7.53	142								
W	17	A	9.5	494	...6	347	50.5	77	38	7.20	7.35	93	6½	50	6 100	5,120	0.2	...6	2.9	Shivering. Quiet.
		B	9.9	355	4..8		32.6	73		5.35	7.35	93								
T	18	A	13.1		520		44.0	82	40	6.05		103	½	50	3,300	1,740	0.1	1.8	1.9	Convulsion.
		B	14.1	356	470	19	44.0	75	38	5.35		146								
M	19	A	19.0	510	670	266	62.0	92	...0			6	8	50	15,200	1..,170	0.9	8.8	9.7	Twitching. Very sick. On verge of convulsion.
		B	21.0	395	422	...04	60.0	...8	28			1..5								
N	20	A	17.4	81	610	412	...8.0	...8	47			93	3½	21	2,220	745				Salivating. Quiet, but not sick.
		B		482	3..5	377	55.0	77	47			95								

* As this is the only experiment in which the cell chloride rose, we suspect the accuracy of our figures in this case

A = Initial values. B = Final values. † = Estimated ‡ = Approximate

Obviously it would be misleading to assume hemodilution in the latter case from the increase in weight alone From postmortem examinations, we are inclined to think that considerable water may be found in the gastro-intestinal tract, which, while increasing the weight of the animal, does not augment the water content of the body tissues or blood

The most consistent change in all reports is the fall in blood chloride From hematocrit, whole blood and plasma chloride determinations, the cell chloride content could be approximated The average initial value for the latter was 335 mgm NaCl per 100 cc of cells, but determinations fluctuated from as high as 450 mgm to as low as 185 mgm In the same group the initial plasma chloride fluctuated from 680 to 498 with an average of 595 The change in cell volume is unrelated to the chloride loss, since the latter may be considerable when there is little change in hematocrit (experiments 11 and 14, Table I) and less marked when there has been quite a drop in cell volume (experiment 2, Table I) These findings are consistent with those of Gram (26), who found no evidence that the chloride concentration in the corpuscles rose or fell with the cell volume

TABLE II

Experiment 1—50 cc distilled water per kilo body weight every half hour from 10 A M to 4 P M

Specimen	Hemoglobin Sahli	CO_2	Whole blood chlorides	Remarks
		volumes per cent		
I, 10 A M	59 5	53	433	
II, 1 P M	54 5	93	409	Vomited at 11 and 12 Salivating but not sick
III, 4 P M	54 0	98	351	Vomited at 1 30 Convulsion at 4 P M

Experiment 1 (Table II) represents one of the first experiments, which followed exactly the technique of Rowntree It is cited here since the enormous increase in bicarbonate led us to suspect alkalosis and to study the loss of chloride more intensively From what has already been said concerning the latter, it is not surprising that analysis of vomitus gives important information While in individual animals time and amount of vomiting vary, there is none the less a definite correlation between the amount of chloride lost by vomiting and the reduction of chloride in the blood In experiment 2 (Table I), where no vomiting occurred, the drop in whole blood chloride was less than in any other experiment, while in experiment 10 (Table I), where no urination occurred, the drop in chloride was large

Rowntree (3) contends that "in dogs, vomiting unquestionably tends to postpone the onset of convulsions" and that in rabbits intoxication

develops earlier because the water is practically all absorbed Our findings, however, suggest that absorption of water is not so important a factor as loss of chloride, particularly by vomiting, and that vomiting therefore hastens rather than delays convulsions As regards rabbits, Gamble (27) has shown that while these animals do not vomit, the stomach dilates and fills with chloride with effects quite comparable to those caused by removal of chloride from the body In the light of Gamble's reports, Misawa's experiments (9), which were limited to rabbits and in which chloride was lowered in all tissues save liver, can easily be explained We believe that a somewhat similar gastric retention without vomiting may have occurred in some of our experiments

Considerable variation in the concentration of chloride in the vomitus can be noted in each of the experiments In general, however, the chloride was more concentrated when the water was retained for longer periods Hence infrequent vomiting might result in as much chloride loss as frequent vomiting of fluid with a lower chloride concentration In some experiments on larger and more resistant animals, as vomiting continued for a long time, there also seemed to be a lessened gastric secretion With regard to the urinary chloride excretion, our findings are at variance with those of Underhill and Sallick (7), and in agreement with those of Rowntree (3) The concentration and quantity of chloride in the urine rapidly fall In fact, the loss by the kidneys usually fails to account for more than about one-tenth of the total loss The loss by vomiting, which has been overlooked by previous investigators, accounts for the fall in blood chloride without the necessity of postulating any peculiar concentration of the ion in tissues

This disappearance of urinary chloride associated with loss of chloride by vomiting is very similar to the findings of Hartmann and Smyth (28) in various types of vomiting in infants Apparently when the level of blood chloride is reduced below a certain threshold little or no chloride is found in the urine No such phenomenon is consistently encountered in the gastric secretion of chloride, though, as previously mentioned, some experiments show a reduction of gastric concentration toward the end of the experiment Figure 2 illustrates by a typical example the changes in urine and vomitus

In some additional experiments on atropinized animals which will probably be considered later, although there was considerable water retention, symptoms of intoxication did not develop, presumably because loss of gastric chloride was minimal

The marked increase in plasma bicarbonate which was found in experiment 1 (Table II) led us to suspect alkalosis The increase was encountered in most of the experiments However in some (experiments 11 12 and 13, Table I) there was a slight reduction Rowntree found the plasma carbon dioxide capacity quite variable the reduction of the alkali

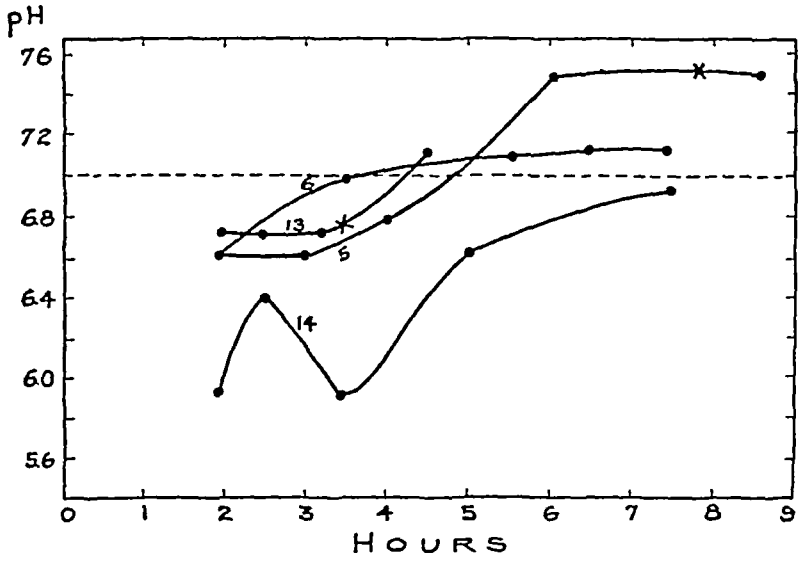

FIG 1 SHOWING CHANGE IN URINARY pH DURING EXPERIMENT
X = Convulsion Figures on curves indicate experiment numbers

HOURS	URINE Vol.cc	URINE NaCl in Sc.	URINE pH	VOMITUS Vol.cc	VOMITUS NaCl in Sc.	VOMITUS pH
	100* discarded	0.02	6.2			
1				500	0.005	5.4
				180	0.002	6.4
2	240	0.003	6.6			
3	140	0.001	6.6	110	0.005	5.4
	115	0.0002	6.8	520	0.004	5.4
				320	0.004	5.4
4	150	0.0002	6.8	220	0.003	5.4
	150**	0.0006	6.6			
5				600	0.002	5.4
	70	0.0006	7.0			
6				420	0.002	6.4
				500	0.002	5.4
	865cc	0.24 Gm. NaCl		3370cc	2.27 Gm. NaCl	

FIG 2 ANALYSIS OF URINE AND VOMITUS IN PROTOCOL 9

* Discarded as urine in bladder before experiment began
** Mixed with small amount of vomitus

reserve observed in some experiments he considered as indicating a loss of alkali

That a different interpretation may be given was apparent when determinations of the hydrogen ion concentration were included In experiments 11 and 13 (Table I), for example, the bicarbonate of the plasma dropped 2 volumes per cent There was, however, a very definite increase in pH In experiments 9 and 10 (Table I) a similar increase was found though the bicarbonate was also increased We feel that the general tendency is toward alkalosis Where the bicarbonate is enormously increased, the pH may change little, but where the bicarbonate is not increased or is reduced, uncompensated alkalosis may be present with a definite change in pH before convulsions

From a clinical standpoint we believe the shallow respiration which is usually present toward the end of the experiment is the typical breathing of alkalosis The manipulation of the animal at the time of venupuncture in many instances upset the respiratory regulation and was perhaps responsible for the uncompensated alkalosis and convulsions The convulsions themselves led to excessive production of lactic acid which to some extent modified the final result.

A study of the pH of urine and vomitus is likewise significant. The vomitus, as would be expected, remains acid In some instances where the water did not remain long in the stomach the acidity was, of course, less marked The pH of the urine, however, definitely increased after the fall in chloride concentration (Figure 1) In experiments 5, 6 and 14 (Figure 1) this is apparent In experiment 13, which represents a rapid development of intoxication, the pH of the urine did not change until after the administration of saline, when it shifted strongly to the alkaline side, pH 7 0 to 7 2 In other words, the excess base of bicarbonate was released when chloride deficiency no longer obtained Sodium chloride supplied base, which had probably been depleted, as well as chloride. The latter replaced bicarbonate, which was in excess, and sodium bicarbonate was excreted

The determinations of total base were unsatisfactory, but the second specimen of serum uniformly showed loss of total base. In experiment 13 the reduction was considerable In this instance also the urine pH did not change until after the therapeutic administration of saline That vomitus may contain varying amounts of duodenal base is probable No attempt was made to estimate this In some instances however, the presence of biliary pigment in the vomitus was obvious, and we are inclined to suspect the fall in total base to be largely dependent on the loss of duodenal contents in the vomitus

With regard to other determinations our findings are similar to those of Rowntree Early experiments included the estimation of diffusible calcium by Greenberg s method (23), but neither this fraction nor the

total calcium was appreciably changed Urea and nonprotein nitrogen determinations were omitted from most experiments for the same reason We had earlier expected that to maintain osmotic pressure non-electrolytes would rise to compensate for the electrolyte reduction The rise in sugar which did occur was associated only with convulsions The rise in lactic acid, which also depended on convulsions, was relatively greater than that of sugar That lactic acid may affect the plasma bicarbonate and play a beneficial role in counteracting alkalosis has already been mentioned

CONCLUSIONS

Neither water retention in the body nor hemodilution is the most essential feature of so-called experimental water intoxication The convulsive symptoms are more closely associated with loss of chloride by way of gastric secretion and a resulting alkalosis

The relative influences of water absorption, chloride loss, and alkalosis have been distinguished by studying the effects of administering varying amounts of water

There is little evidence that non-electrolytes increase in the blood to compensate osmotically the electrolyte depletion

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28 Hartmann A F and Smyth F S , Am J Dis Child , 1926, xxxii 1 Chemical Changes in the Body Occurring as the Result of Vomiting

STUDIES ON GALLBLADDER FUNCTION IX THE ANION-CATION CONTENT OF BILE FROM THE NORMAL AND INFECTED GALLBLADDER

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In an earlier paper (1) we discussed the anion-cation concentration of normal hepatic bile when subjected to the activity of the dog's normal gallbladder Under these conditions the chloride and bicarbonate concentrations and the pH of hepatic bile decrease, while the base, bile salt and calcium concentrations increase In every instance fluid is absorbed In a very limited number of observations we have found the depression of the freezing point of hepatic and gallbladder bile approximately the same, although the total anion-cation concentration was increased considerably in the latter

As a prelude to the anion-cation studies we had studied the effect of gallbladder activity on certain of the individual constituents of hepatic bile when placed in the normal bile free gallbladder of the unanaesthetized dog (2) (3) (4) It was observed that when the gallbladder became infected the activity of the membrane on the constituents studied was altered considerably In such instances, water was only slowly absorbed, or, in the more severely damaged organ, fluid actually poured into its lumen, the latter action being a complete reversal of the normal mechanism Under these circumstances chloride and bicarbonate entered the gallbladder lumen with the inflowing fluid, the chloride concentration of the secreted fluid being about plasma level while the total CO_2 concentration was often several times the normal plasma level Calcium, introduced as calcium lactate, was precipitated, partly in the gallbladder lumen, and partly in the gallbladder wall Cholesterol in either a colloidal suspension, or in hepatic bile, increased in concentration, but not in total amount when placed in the normal gallbladder The same was true of bile pigment. When, however, the gallbladder was infected the total amount of cholesterol increased, while the total amount of bile pigment decreased (5)

These isolated observations made at a time when we were chiefly concerned with normal function convinced us that a study of the anion cation changes in hepatic bile subjected to the activity of an abnormal

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gallbladder was necessary in order to interpret the changes which occur in disease of that organ

METHOD

The initial studies were made on the dog using a preparation similar to that which we have previously described (2), except that after convincing ourselves that the gallbladder was functioning normally, we either intentionally or unintentionally permitted the gallbladder to become infected The usual infecting organism was a non-hemolytic streptococcus In the studies on the dog, known amounts of normal dog's hepatic bile were placed in the infected bile-free gallbladder After varying intervals the bile was recovered and the various constituents of the bile were determined by the methods reported in previous papers (1) (2) (3) (4) (5) The dogs behaved normally, and beyond the infection of the gallbladder, no evidence of disease existed

In the human cases the bile was removed from the gallbladder at the operating table It was transferred to a test tube aseptically and the analyses were made as soon as it was possible to transfer the bile to the laboratory Some of the patients were operated on under local anaesthesia, some under spinal, while others received nitrous oxide and oxygen plus a small amount of ether

Some studies have been made on liver bile obtained after drainage of the common duct for obstruction The bile specimens so obtained were collected in receptacles for 24 hours when samples were removed for analyses Successive samples were analyzed until evidence was obtained that the liver bile was approaching what we thought was a normal liver bile, or until the surgeon removed the common duct drainage tube, making further specimens unobtainable

TABLE I

Analysis of the gallbladder bile from the normal and infected gallbladder of the dog

Dog number	Date	Bile in	Bile out	Bile removed					
				Base	Calcium	Chloride	$BHCO_3$	Bile salt	pH
		cc.	*cc.*	*m eq per liter*	*m eq per liter*	*m eq per liter*	*m eq per liter*	*m eq per liter*	
490	* November 23–24, 1931	96 5	22 0	240	31 1	2 1	6 7	252 0	
	† January 26–27, 1932	14 0	29 0	184	5 2	111 2	47 2	2 1	7 5
	† January 31–February 2, 1932	5 0	29 0	184	6 6	129 3	37 4	0 0	7 8
410	* November 17–18, 1931	57 0	8 8	241	26 4	8 2		282 0	
	† November 20, 1931	10 0	28 0		11 2	78 9	52 5	12 9	
203	* September 21, 1931	23 0	8 0		17 1	37 2	7 3	168 4	6 6
	† September 22, 1931	30 0	34 0	169	7 0	85 4	40 2	22 4	7 5

* Gallbladder normal
† Gallbladder infected

RESULTS

In Table I are given data from several animals before and after gallbladder infection had taken place. In the normal gallbladder the concentration of chloride and total CO_2 decreased very markedly, as did the total fluid volume Since hepatic bile was added at intervals to the gallbladder contents the relation of time to total fluid introduced and removed was quite variable The volumes reported merely indicate direction rather than rate of change With a single injection of bile we have found that the gallbladder will concentrate the fluid volume in a 24 hour period to as little as one-sixteenth the original amount introduced Furthermore, in the normally functioning gallbladder the pH of the bile uniformly decreased, while the base, bile salt and calcium concentrations increased

When the gallbladder became infected there occurred a nearly complete reversal of its activity The chloride and total CO_2 concentrations were uniformly higher In the infected gallbladder the pH of the bile increased In contrast, the base, bile salt and calcium concentrations decreased The tremendous loss of bile salt under such conditions is indeed striking Fluid volume, instead of decreasing, increased, indicating not only a failure of absorption but an actual pouring of fluid into the gallbladder lumen Since no bile could enter the gallbladder except that which was placed in it the additional fluid must have come from the vessels of the gallbladder wall

The data from the human gallbladder bile fall into several groups The specimens were analyzed, without our knowledge in many instances of the clinical findings After analysis the surgeon was asked for the roentgenologic and operative findings, and it was soon observed that the data were roughly divisible into several groups

No data were found in the literature based on analyses of normal human gallbladder bile It was important to obtain such data if a comparison was to be made with the dog s gallbladder bile We secured one specimen of gallbladder bile by aspiration from a patient whose abdomen was opened under local anaesthesia and a second specimen was

TABLE II

Comparison of normal human and dog s gallbladder bile

Dog number	Base	Calcium	Chloride	Bile salt
	m eq per liter	*m eq per liter*	*m eq per liter*	*m eq per liter*
410		22 4	10 3	188 8
472		25 5	3 9	304 0
446		27 6	4 7	289 0
Patient				
D	298	28.5	16 9	208 0
E.		25 9	18 4	152.0

supplied to us by Dr E L Eliason from a patient operated on under nitrous-oxide-oxygen-ether anaesthesia (Table II) Neither of these cases had any evidence of biliary tract disease These gallbladders at operation were thin and blue, and free of any adhesions It will be readily seen that there is a fairly close parallelism between the normal gallbladder bile of man and dog, as regards the constituents which we are reporting

TABLE III

Analysis of gallbladder bile of human cases Chronic cholecystitis—no stones Gallbladder visualized after administration of sodium tetraiodophenolphthalein

Patient	Base	Calcium	Chloride	Bile salt
	m eq per liter	*m eq per liter*	*m eq per liter*	*m eq per liter*
R		12 1	57 6	99 8
S	290	24 0	17 1	99 3
D	263	15 2	41 8	79 0
B	220		69 3	62 0

TABLE IV

Analysis of gallbladder bile of human cases Chronic cholecystitis—with stones Gallbladder visualized after administration of sodium tetraiodophenolphthalein

Patient	Base	Calcium	Chloride	Bile salt
	m eq per liter	*m eq per liter*	*m eq per liter*	*m eq per liter*
E		18 3	39 5	81 0
G		20 8	52 0	78 3
A	189	12 1	68 1	52 4

TABLE V

Analysis of gallbladder bile of human cases Chronic cholecystitis—with stones Gallbladder not visualized after administration of sodium tetraiodophenolphthalein

Patient	Base	Calcium	Chloride	Bile salt
	m eq per liter	*m eq per liter*	*m eq per liter*	*m eq per liter*
P		10 7	115 6	52 0
Be	199		63 6	42 0
C		9 5	60 9	32 3
M				26 0
Br	151	3 0	140 7	3 4
L		4 5	93 5	1 8
H	151	10 0	112 7	0 0
T	121	5 3	80 1	0 0
S	146	7 2	103 4	0 0
W		1 6	152 2	0 0
Sa			81 1	0 0
Mc.		2 8	82 5	0 0

In Tables III, IV and V are given data from specimens of pathological human gallbladder bile In the cases in Groups III and IV the gallbladder was visualized after administration of sodium tetraiodophenolphthalein Visualization was not obtained in Group V It is difficult to state quantitatively the degree of visualization since the technics employed in the administration of the dye may have varied By visualization we mean that the roentgenologist was able to see the gallbladder outline, but this may or may not have been considered entirely normal

As a whole these data show a decrease in the calcium concentrations, the lowest concentrations occurring in the non-visualized gallbladders The chloride concentration shows progressive increase while the bile salt concentration tends to become lower as we pass through Groups III and IV to Group V, until in the latter group there are a number of instances in which no bile salt could be demonstrated The base concentration decreases, the greatest reduction being found in Group V

The surgeons' findings and the pathologists' diagnoses in the cases reported in Tables III, IV and V were in all instances in agreement. In Group III the gallbladder was slightly thickened and opaque may or may not have had adhesions about it and did not contain calculi The pathologic findings were those of chronic interstitial cholecystitis The findings in Group IV were similar to those in Group III except for the presence of calculi In Group V the evidences of cholecystitis were more marked and calculi were always found

TABLE VI

The time interval from release of an obstruction of the common bile duct to the reappearance of the typical Gregory-Pascoe reaction in liver bile

Patient	Cause of the obstruction	Time interval to reappearance of bile salts *days*
B	Calculus	16
S	Calculus	27
J	Calculus	(16 *)
K	Calculus	12
D	Calculus	25
St	Stricture	15
A.	Stricture (partial)	8
W	Carcinoma of pancreas	(14 *)
Sch	Suppurative cholangitis	(15 *)

* Unable to obtain bile after this period no Gregory Pascoe reaction in last specimen obtained

In some cases, as the result of an obstruction of the common bile duct hepatic secretory suppression had occurred After the release of the obstruction the bile began draining from the liver and rapidly became what we have reason to believe is normal except for one feature For a

considerable period, frequently as long as three weeks, the typical color of the Gregory-Pascoe reaction for bile salt was not developed Instead of the normal blue color, which this method develops, a variety of shades from red to blackish purple were obtained As the drainage was continued some bile salt made its appearance, but this occurred only late It would appear that when the liver cells have been damaged during obstruction, the bile salts are for a time excreted either not at all, or at least not in their usual form In Table VI are given the intervals before reappearance of the typical Gregory-Pascoe reaction for bile salts in a few of these cases

DISCUSSION

We believe that these analyses of normal human gallbladder bile are the first which have been reported Hammarsten (6) and others have published data on human bile but the material was either obtained at autopsy or from patients operated on for biliary tract disease, a condition which we have shown greatly alters the anion-cation concentration of gallbladder bile We are unable to offer any data on normal human liver bile Obviously drainage of the common duct in a patient with no evidence of a lesion of the biliary tract or pancreas is not justifiable

We have, however, continued the study of hepatic bile from patients after relief of an obstruction of the common duct, until the concentration of the constituents studied became nearly constant from day to day

In a previous paper we reported that hepatic bile of the dog is more variable in its electrolyte composition than is serum (1) The same appears to be true for the hepatic bile of man After long continued drainage of the common duct, so that the hepatic bile was as normal as we had reason to believe it might become, base varied from 151 to 181 m Eq per liter The chloride concentration varied from 76 to 110 m Eq per liter, the majority of the figures being below the plasma level for chlorides This is also true of the hepatic bile of the dog The calcium concentration of human hepatic bile was found to vary from 1 9 to 10 2 m Eq per liter In the majority of instances the concentration was higher than that of plasma, a relationship which is also true of the dog The bile salt concentration was even more variable, concentrations from 3 3 to 52 6 m Eq per liter being obtained These data, as a whole, are not unlike those of the dog and although we do not present them as derived from normal human hepatic bile, they are at least indicative of the general trend of its anion-cation concentrations From these studies we are of the opinion that the hepatic bile of man and dog are similar with respect to the concentrations of the constituents which we are reporting in this paper

The data on the bile obtained from the normal human gallbladder are quite similar to those from the dog We have not measured bile pigment or cholesterol in the human cases except in isolated instances, so that at

present we are unable to compare the concentration of these components in dog and human bile Our few data are not inconsistent with the view that the cholesterol concentration of human bile is higher than that of dog's bile

It would seem that the more severely damaged the gallbladder the more widely do the concentrations of the constituents under consideration vary from those found in normal gallbladder bile As long as the gallbladder can be visualized after the administration of sodium tetraiodo phenolphthalein, base, calcium and bile salts become concentrated, while chloride is absorbed As clinical and pathological evidence of change in the gallbladder became more definite the composition of the gallbladder bile approached that characteristic of hepatic bile In the occasional case (Be. and C , Table V) evidence of some concentration or absorption of the appropriate constituents was found even though the gallbladder was not visualized The progressive decrease in the bile salt concentration with increasing evidence of damage was the outstanding finding in these studies Closely paralleling this was the rise in the chloride concentration Ravdin, Morrison and Smyth (7), Newman (8), and Andrews, Schoenheimer and Hrdina (9) have previously pointed out that the bile salt concentration of the bile from the damaged gallbladder is low The first authors found that the concentration of bile salt in gallbladder fistula bile in the presence of a diseased gallbladder was lower than the bile salt concentration in common duct fistula bile Whether the loss of bile salt is due to absorption or change in its structure we are unable to state

The decrease in the calcium concentration with increasing evidence of gallbladder damage is of interest. No cases in this series were of the type reported by Phemister, Rewbridge and Rudisill (10), where undoubtedly the calcium concentration of the material found in the gallbladder was high Those cases, however, had complete or incomplete cystic duct obstruction while the studies reported in this paper were made upon subjects with the cystic duct patulous Whether the low calcium concentration is merely that of hepatic bile or whether it is further lowered due to absorption, precipitation, or dilution is as yet unknown The rise in the pH and the increase in the total CO_2 concentration of gallbladder bile after gallbladder damage presumably offer favorable conditions for precipitation of calcium Against the probability of dilution is the observation that in the dog when quantitative studies were made calcium was actually lost from the fluid contents of the gallbladder

It may well be, however, that the severely damaged gallbladder of man secretes fluid into its lumen as it does in the case of the dog If this is true the sodium tetraiodophenolphthalein, which after administration is secreted in the liver bile, may be further diluted when it comes to the gallbladder Thus, what the roentgenologist frequently reports as a

failure of the gallbladder to concentrate, which would imply a static condition, may represent actually a process of active dilution

The complete absence of bile salt from liver bile obtained shortly after the release of an obstruction which has caused complete hepatic secretory suppression, and its gradual reappearance as the flow of bile continues unimpeded, may be considered as suggestive of the synthesis of bile salts in the liver The period of time necessary for the reappearance of bile salts, as demonstrated by the typical Gregory-Pascoe reaction, further substantiates the view held by some clinicians that complete recovery of hepatic function is not a rapid process

Accurate records of the amount of bile obtained daily from a "T" tube in the common duct after the release of an obstruction have been kept in the cases studied In a subsequent paper we plan to discuss the loss of organic and inorganic salt when the drainage is continued over a long period

SUMMARY

The concentrations of base, calcium, chloride, and bile salt in the bile from the normal human and dog's gallbladder are reported The changes in concentration of these substances when the human or dog's gallbladder is diseased are also reported There appears to be a definite relationship between the extent of the gallbladder damage and the degree to which the activity of the organ is altered from the normal

Of the constituents which we have studied bile salts and chloride show the more constant changes in disease of the gallbladder The more severely damaged the gallbladder the lower the bile salt and the higher the chloride concentrations In the dog, total CO_2 increases in concentration in disease of the gallbladder and presumably this is also true for man

There is a fairly close correlation between extent of disease, visualization after the administration of sodium tetraiodophenolphthalein and the deviation of the chemical findings in the bile from the normal

For a number of days after the release of an obstruction of the common bile duct, bile salt, as determined by the typical Gregory-Pascoe reaction, fails to make its appearance When the liver bile returns to what we suppose to be normal the concentrations of base, calcium, chloride, and bile salt are not unlike those found in the normal liver bile of the dog

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THE UNAVOIDABLE ERROR IN THE DIFFERENTIAL COUNT OF THE LEUKOCYTES OF THE BLOOD

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In differential blood counting, one attempts by careful observation of a limited number of leukocytes to obtain a picture of the actual distribution of the various types of cells in the blood stream Many physicians are inclined to accept a count done by a competent person as a very close approximation to this value There are many statements in the literature which indicate an absolute confidence in the results of differential counts of which the following quotation from an article on surgical lesions of the abdomen is a typical example. "Probably the blood count is of the greatest value in determining the severity of the lesion in acute appendicitis When the polymorphonuclear percentage is 70, or below, a suppurative or gangrenous process may be ruled out and operation delayed or postponed with safety It is difficult to be sure of the severity of the process when the percentage of polymorphonuclears is between 70 and 80 I have seen beginning peritonitis with percentages below 80 but above this point one may feel sure that he is dealing with a dangerous lesion which should have immediate treatment"

Such confidence is unwarranted, since differential counting of the leukocytes is one of the most uncertain of the quantitative methods used in medicine There are three types of error in this procedure The first is the mechanical error, which includes all variations in taking the blood sample, making the smear and staining, and all irregularities in distribution of the cells depending upon the type of smear and the areas over which the count is made The second type is the error of interpretation, the magnitude of which depends entirely upon the judgment of the observer The third is the error due entirely to chance, which is the subject of the present paper

Many articles have been written on the errors of blood counting, but most of them are concerned with the mechanical errors Thus, Napier (1), Meissner (2), Stephens et al (3), Beacom (4) and Gyllenswärd (5) describe variations in differential counts on smears made in various ways the counts being made over various areas of the smears No two of these authors agree as to the most accurate method of making the count The chance errors either are not mentioned or are discussed very briefly All

agree, however, that at least 300 cells must be counted if the results are to be at all accurate

Dahlberg (6) attempts to determine the magnitude of the various types of error and gives variabilities ± 4 3 per cent for the mechanical error, ± 11 per cent for the error of interpretation and ± 5 8 per cent for the chance error on counts of 500 cells The data used as a basis for these results are not given, and the exact meaning of the results is not clear Kilgore (7) calculated the chance error on a count of 100 cells and found that with an actual count of 50 per cent lymphocytes, different observations on the same blood would be expected to vary between 35 per cent and 65 per cent He did not check the calculation with any actual counts

Brandt (8) studied the mechanical and chance errors in red, total white and differential white blood counts He calculated that in counting 500 cells with 66 per cent of polymorphonuclears, the standard deviation would be 2 11 per cent of the total count or 3 2 per cent of 66 He compared this with six counts of 500 cells each in which he obtained a standard deviation of only 1 4 per cent of 66 Six, however, is far too small a number of counts for an accurate determination of the standard deviation

An error due entirely to chance occurs whenever in dealing with a universe composed of a very large number of individual units our estimate of the distribution of different types of units must be based upon the study of an extremely small proportion of them Thus in differential counting we study a few hundred cells in order to determine the proportion of the various types in the entire blood stream, which, if we assume a white count of 10,000 cells per cubic millimeter, and a blood volume of 5 liters, will contain 50,000,000,000 cells It is obvious that when from such a tremendous total number of cells, samples as small as one hundred are taken, each will differ slightly from another, and no single count will give a true picture of the actual distribution This error is completely independent of technique or interpretation and is entirely unavoidable It can be decreased by increasing the number of cells studied in the total count, but it cannot be eliminated, and when small numbers of cells are counted, it leads to a large variation as will be shown

Even when the same blood is subjected to a series of counts, there will be a certain variation or dispersion in the results The simplest method of expressing the magnitude of this dispersion is to note the range between the highest and lowest values obtained for a particular type of cell This method is misleading, however, because a single count that happens to be far above or below the others will be unduly weighted The commonest and most satisfactory expression of the magnitude of the dispersion is obtained by dividing the sum of the squares of the differences between the individual observations and the mean value by the total

number of observations and taking the square root of the result This measure of dispersion is called the standard deviation

If the standard deviation can be determined, it is easy to estimate the amount of variation to be expected as the result of chance alone The chances are approximately 2 to 1 that a particular observation will fall within the bounds of one standard deviation above or below the actual value, about 20 to 1 that it will fall within twice the standard deviation, and about 370 to 1 that it will fall within three times the standard deviation above or below the true value It is generally considered that chance errors may be as great as three times the standard deviation above or below the actual value

In differential blood counts, the standard deviation can be determined readily according to the formula, from Yule (9), page 257

$$\sigma = \sqrt{Npq}$$

σ = the standard deviation,
N = the number of cells counted,
p = the proportion of cells of a certain type,
q = the proportion of all other types of cells

This formula gives the standard deviation in per cent of the total count Thus if we count 100 cells with 25 per cent of lymphocytes and wish to find the standard deviation, we will have

$$N = 100,$$
$$p = 25,$$
$$q = 75,$$
$$\sigma = \sqrt{100 \times 25 \times 75} = 4\ 3$$

We would expect chance errors to give a variation of three times this value above and below the actual value of 25 per cent. Three times 4 3 is 12 9, consequently a single count on 100 cells might give a lymphocyte percentage anywhere from 12 1 per cent to 37 9 per cent Similar calculations will give the expected variations at all percentages from 0 to 100

This chance variation is not entirely theoretical, and it can be checked by actual counts For this purpose, 100 differential counts were done on the same blood A sample of blood was taken with a little sodium citrate to prevent immediate coagulation, and fifty smears were made by the cover glass method as rapidly as possible These were stained and those with eight or more satisfactory low power fields were selected On each smear, six counts of 100 cells each were made consecutively, all the counts being done in areas where the red cells were just separated from each other Since all the smears were made with the same blood and in the same way, it was hoped that the mechanical error would be eliminated or

would be of about the same magnitude in each count All the counts were made by one individual, which should make the error of interpretation negligible The observed variations should therefore be almost entirely the result of chance

The present work is concerned only with the chance distribution of observations about certain mean percentages, and consequently the actual type of cell in any group is of no significance Therefore, any two or more types may be taken together to form a single larger group, providing the combination is made in each individual count Several such groups were made in order to increase the range of observations from 50 per cent to 100 per cent To avoid repetition of the names of the different cell types, they will be designated by letters as follows

a = basophiles,
b = eosinophiles,
c = large mononuclears,
d = lymphocytes,
e = polymorphonuclears,
g = polymorphonuclears + lymphocytes,
h = polymorphonuclears + lymphocytes + large mononuclears,
i = polymorphonuclears + lymphocytes + large mononuclears + eosinophiles

Occasional atypical cells were found that could not be placed in any of the above classes The number of these was noted to preserve the true percentages of the other types, but they were not included in the calculation

When the counts were completed, frequency tables were prepared for each type of cell For all types except a, b, and i, the dispersion was large enough so that the listing of each individual percentage in which an observation happened to fall would make a table that contained too many classes Therefore, the percentages were grouped in pairs, with a class interval of 2 per cent, and the number of observations that fell into each class was counted and listed in a frequency table under f The means and standard deviations were calculated from the tables in the usual way (cf Yule, pp 110 and 134)

Table I is a frequency table for cells of type e It is given in full to indicate the method of calculation Table II is a summary of the means and the theoretical and observed standard deviations for each type of cell

Examination of the tables shows that in every case there is a close agreement between the standard deviation calculated for the percentage of cells in question and the actual standard deviation of the observed values In other words, the dispersion observed in a series of 100 differential counts was just what was to be expected from calculation of the theoretical dispersion In nearly every case, the observed standard

deviation was slightly higher than the theoretical one. This is obviously due to the fact that the complete elimination of mechanical errors was impossible, but the close agreement demonstrates that they were reduced to a very small value

TABLE I

Frequency table for cells of type e

Class	f	x	fx	fx^2
36–	1	−14	−14	196
38–	1	−12	−12	144
40–	1	−10	−10	100
42–	7	−8	−56	448
44–	5	−6	−30	180
46–	10	−4	−40	160
48–	16	−2	−32	64
50–	15	0	0	0
52–	18	+2	+36	72
54–	12	+4	+48	192
56–	4	+6	+24	144
58–	6	+8	+48	384
60–	4	+10	+40	400
	100		+2	2484

$M = 50\,5 + 2/100 = 50\,52.$
$\Sigma fx^2/N = 2484/100 = 24\,84$
$\sigma^2 = 24\,84 - \overline{02}^2 = 24\,84$
$\sigma = 4\,98$
$\sigma_1 = \sqrt{100 \times .51 \times .49} = 5\,0$

Theoretical standard deviation = 5 0
Observed standard deviation = 4 98

TABLE II

Table showing the means and standard deviations of the different types of cells

Type of cell	Mean M	Range of observed values	Observed standard deviation	Theoretical standard deviation
			σ	σ_1
a	0.52	0–3	0 69	0 71
b	2.59	0–8	1 67	1 59
c	16 62	7–27	4 22	3 67
d	28 02	14–38	5 05	4 49
e	50 52	37–60	4 98	5 00
g	78.50	63–87	4.59	4 14
h	94 42	87–99	2 64	2.37
i	96.58	91–100	2 03	1 81

All figures in this table are expressed in per cent of the total count

In Chart I, the individual observations on the percentages of groups *d*, *e* and *g* have been plotted to show the distribution of the counts in relation to multiples of the standard deviation The actual percentage

on any one count is obtained from the chart by adding to the mean value for the particular type of cell the deviation as measured on the vertical axis The mean value is indicated on the horizontal axis by a vertical line drawn through the group of spots

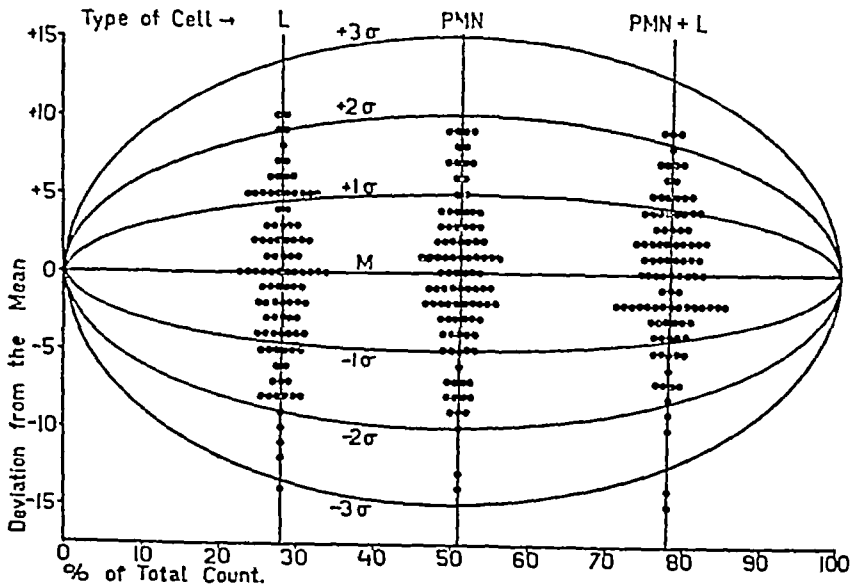

CHART 1 CHART SHOWING THE DEVIATION OF THE INDIVIDUAL COUNTS FROM THE MEAN, IN RELATION TO MULTIPLES OF THE STANDARD DEVIATION

The distribution of observations with relation to the standard deviation is very close to the expected one If chance alone is responsible for the deviations, and if a large enough number of observations is made, 68 3 per cent of the observations will lie within one standard deviation of the mean, 95 4 per cent within two and 99 7 per cent within three In the chart it will be seen that three observations of the 300 shown lie outside the line representing three times the standard deviation This is more than would be expected from chance alone, but again it is probably the result of an added mechanical error

DISCUSSION

The results show that the completely unavoidable error in differential blood counts, which is due to chance, and which cannot be eliminated by the most perfect technique, may be of considerable magnitude It may be decreased by counting larger numbers of cells, and Chart 2 shows the maximum chance errors to be expected with differential counts done on 100, 200, and 400 cells It is to be noted that the error is inversely proportional not to the number of cells counted but to the square root of the number In other words, in order to double the accuracy of a count

it is necessary to count four times the number of cells, and to triple it, nine times

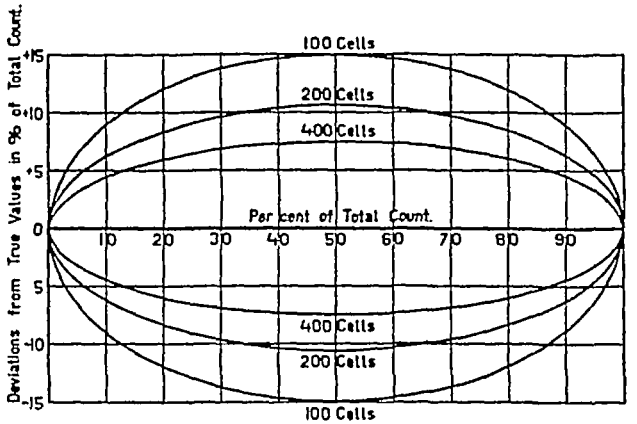

CHART 2 CHART SHOWING THE MAXIMUM ERRORS DUE ONLY TO CHANCE IN DIFFERENTIAL BLOOD COUNTS WITH THE TOTAL COUNTS OF 100, 200, AND 400 CELLS

Another question which may at times be of considerable importance is that of the significance of a change in the proportion of a certain type of cell in two different counts Thus suppose we have a patient suspected of acute appendicitis, whose differential count of 100 cells shows 70 per cent of polymorphonuclears, and an hour later, 80 per cent Is this a significant change, or may it be due entirely to chance variation? This is decided by determining the standard error of the difference of the two counts according to Yule, page 268 If the observed difference is less than three times the standard error of the difference it may be entirely the result of chance The method of calculation is as follows

$$p_0 = \frac{n_1 p_1 + n_2 p_2}{n_1 + n_2},$$

$$e_{12}^2 = p_0 q_0 (1/n_1 + 1/n_2),$$

where n_1 and n_2 are the numbers of cells counted in the two counts p_1 and p_2 the proportion of polymorphonuclears in the two counts and e_{12} the standard error of the difference

In the example just cited n_1 and n_2 each equal 100 and p_1 and p_2 are 70 and 80 respectively Therefore,

$$p_0 = \frac{100 \times 70 + 100 \times 80}{200} = 75,$$

$$e_{12}^2 = 75 \times 25(1/100 + 1/100) = 00375,$$

$$e_{12} = 0613 \text{ or } 6\ 13 \text{ per cent}$$

The observed difference of 10 per cent between the percentage of polymorphonuclears in the two counts is about one and a half times this standard error and thus cannot be considered significant

It is apparent that differential blood counts done on 100 cells, even with the most perfect technique and interpretation, are subject to such wide fluctuations as a result of chance variations in distribution that they are practically without significance When to this unavoidable error there is added the mechanical error and that of interpretation, the result must necessarily be extremely unreliable As has been repeatedly pointed out by others, at least 400 cells must be counted before the results of a differential count may be considered at all reliable and even here the maximum chance error may be as much as 7 5 per cent of the total count, as is shown in Chart 2

To judge the accuracy of a particular count, it is only necessary to read from Chart 2 the deviation to be expected for a certain observed percentage of cells on the curve corresponding to the number of cells counted This deviation added to and subtracted from the observed value will give an estimate of the range within which the true value will probably lie Thus if a lymphocyte percentage of 40 is observed in a count of 200 cells, the vertical line through 40 per cent on the horizontal axis is followed up and down until it intersects the curve marked 200 cells The points of intersection are at about plus and minus 11 per cent Consequently the true percentage of lymphocytes may be expected to lie between 29 per cent and 51 per cent

SUMMARY

1 The error in differential blood counts due to chance variations in distribution is pointed out, and its magnitude for counts done on various numbers of cells is calculated

2 One hundred differential counts of 100 cells each on the same blood are reported, and the dispersion of the separate observations is shown to agree closely with the predicted value

3 It is concluded that at least 400 cells must be counted before the results of a differential count may be considered reliable, and even here the maximum error due entirely to chance is 7 5 per cent of the total count

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THE ADDIS SEDIMENT COUNT IN NORMAL CHILDREN

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THE METHOD

In 1925 Addis (1) described a method by which, in a concentrated acid urine, the rate of excretion of protein, casts and red and white cells could be determined His method, with certain modifications, has been followed here All of the counts were made on the 12 hour night specimen from 7 or 8 P M to 7 or 8 A M Addis recommended that fluids be restricted during, and for 12 hours preceding the collection, since in dilute and alkaline urine hyaline casts dissolve and red cells may be completely lysed With children this rigid restriction of fluid proved impossible. Withholding fluid during the afternoon and night except for 200 cc at the evening meal, gave urines of such concentration and acidity that they were suitable for a count Most children had an early supper and collections were started at 7 or 8 P M Under these conditions, the urinary pH was between 5 0 and 6 0 and the specific gravity usually well above 1 020 The specimens were treated as described by Addis "the concentrated night urine is thoroughly mixed by repeated inversion of the rubber-stoppered bottle and a 10 cc. sample is transferred to a special graduated tube, and centrifugalized for five minutes at 1,800 revolutions per minute. The supernatant urine is decanted and pipetted down to a known volume which varies with the amount of sediment as judged by direct observation The casts and cells are thoroughly mixed in this remaining fluid by means of a capillary pipette and drops are transferred to a hemocytometer The casts are counted under the low power microscope in several unit volumes of 0 0009 cc., the number depending on the concentration The cells are counted in unit volumes of 0 0001 cc with a 1/6 eyepiece of number 10 objective The calculation is very simple if a slide rule is used The rates of cast red blood cell and white blood and epithelial cell excretion are expressed always as twelve hour rates.' For the quantitative protein the method of Shevky and Stafford (2) was followed with a modification in calculation suggested by Addis. In this method the volume of protein precipitated by a solution of phosphotungstic acid in strongly acidified alcohol is determined the precipitate being packed by centrifugalizing under constant conditions

161 sediment counts on 74 normal children

Children admitted to the hospital for hernia and eye operations or for observation for various conditions were considered normal when the routine urinalyses were negative Cases in which there was any possibility of renal irritation were not included Addis thinks that for cell counts in women, catheterization is essential In this series no precautions were taken to secure unusual cleanliness, though cases of vaginitis were not included A number of counts were done on children who were not under hospital care and whose diet and activity, therefore, could be considered more normal No differences were observed in the counts The age range was from 4 to 12 years Children in the lower age groups (4 to 7 years) gave slightly lower figures for protein, casts and cells than the older children, and zero counts were more frequent in this group The excretion of epithelial and white blood cells was found to be somewhat higher in female than in male children

The results are shown in Table I, the range and averages are shown in Table II The series is small and the distribution is so skewed that no statistical analysis has been made The probable error of the means has been computed In Table III tentative figures are given marking the upper limit of normal excretion in children from 4 to 12 years of age

TABLE I

12 hour excretion of protein, casts and cells 161 observations on 74 normal children 4 to 12 years of age

Protein		Casts		Red blood cells		Epithelial and white blood cells	
Mgm.	Number of observations	Thousands	Number of observations	Thousands	Number of observations	Millions	Number of observations
0	0	0	118	0	108	0	0
1 to 5	8	Over 0 to 2	9	Over 0 to 20	11	Over 0 to 5	133
6 to 10	30	Over 2 to 4	17	Over 20 to 40	12	Over 5 to 1	19
11 to 15	40	Over 4 to 6	9	Over 40 to 60	14	Over 1 to 1 5	4
16 to 20	28	Over 6 to 8	3	Over 60 to 80	11	Over 1 5 to 2	0
21 to 25	13	Over 8 to 10	2	Over 80 to 100	3	Over 2 to 2 5	2
26 to 30	16	Over 10 to 12	2	Over 100 to 120	1	Over 2 5 to 3	3
31 to 35	13	Over 12 to 14	1	Over 120 to 140	1		
36 to 40	8						
41 to 45	4						
46 to 50	1						

TABLE II

12 hour excretion of protein, casts and cells Range and average in normal children and adults

		Children	Adults (Addis)	Adults (Goldring)
Protein, *mgm*	Range Average	3 to 47 18 5±.5		3 to 60 14 6
Casts	Range Average	0 to 12 916 1 085±123	0 to 4,270 1,040	0 to 9 200 647
Red blood cells	Range Average	0 to 129 000 15 181±1 400	0 to 425 000 65,750	0 to 1 530 000 163 000
Epithelial and white blood cells	Range Average	9 000 to 2,822 000 322 184±25 500	32 400 to 1,835 000 322,500	24,000 to 3 400,000 647,500

TABLE III

The upper limit of normal excretion per 12 hours in children from 4 to 12 years of age

Protein	35 mgm
Casts	10 000
Red blood cells	600,000
Epithelial and white blood cells	600 000 (male) 1,000 000 (female)

Comparison with adult normals

Protein Addis (3) says 'through observations on many normal individuals and on many patients who were passing from what we call Bright's Disease back to normality,' we have reached the tentative conclusion that the upper limit of normal variation is in the neighborhood of 30 mgm of protein per 12 hour period" Our normal range goes somewhat higher than this, i e , 47 mgm and Goldring and Wyckoff (4) in 21 adult normals found a range of from 3 to 60 mgm It is not surprising that our average and normal range is higher than in the adult series Albuminuria in healthy children is a common finding from the age of six to puberty and is very common during puberty

Casts As in the adult series of Addis, practically all of the casts were hyaline The upper normal limit is higher than that given by either Addis or Goldring but the average is the same Thirty-eight of 161 cast counts were above the average, while 118 or 73 per cent were zero counts In Addis s series there were 39 of 74 cast counts above average and 40 per cent zero counts Possibly the high percentage of zero counts in the children of this series is due to the fact that fluids were not rigidly restricted

Red blood cells The presence of red cells in the urine is frequently regarded as indicative of some abnormality in the urinary tract. The work of Addis and others has shown that normal adults may excrete

small numbers of red cells in the urine That this also holds for normal children is evident from the studies presented here Red cells are not constant urinary constituents In some children they have been found at one examination and not at another, and, in others, repeated examinations have failed to disclose their presence

Our average (15,181) is lower than that of Addis (65,750), and our percentage of zero counts (67 per cent) is higher than that of Addis (51 per cent)

Several factors may account for the lower red cell excretion in children It is conceivably the result of failure to restrict fluids rigidly It may be related to difference in kidney weight While the child has the same number of glomeruli and tubules as the adult, it is obvious that they must be smaller Addis has found that the actual red cell excretion may be obscured at times when the salt concentration in the urine becomes so low that red cells are invisible Children, and especially hospital children, have a very low salt intake Three of our normal children who had been showing zero red cell counts were on two occasions given 5 grams of salt at the evening meal In all of these the red cell counts remained zero On the whole it seems fair to assume that the relatively simple life of the child, in which stresses, strains and complicating features (latent infection, local disease of the urinary tract) are correspondingly few, is a factor of some importance in determining the lower erythrocyte excretion

White blood cells and epithelial cells The average for children is practically the same as that for adults The high range and average in this series is probably due to the fact that we did not catheterize female children The average for male children is about half that of the adult group

The sediment count compared with routine urinalysis

The results of 51 sediment counts have been compared with the reports from the routine microscopic examination of the same specimen Ten cc of the thoroughly mixed urine were used for both procedures The specimen for routine examination was centrifuged for the same length of time and at the same rate of speed as in the quantitative method The only difference was that in the routine examination the sediment was examined practically undiluted, while in the count the sediment was always mixed in from 1 to 3 cc of urine More than the usual time and care were taken for the routine examination Cases were chosen where the volume was approximately the same (about 200 cc) and where the changes were slight or moderate They were cases of acute infection, subsiding nephritis and rheumatic carditis

Protein Table IV indicates a relatively close agreement between quantitative protein and qualitative albumin reports With percentage values up to 01 per cent, the qualitative tests were reported "negative"

or "trace," and with urine volumes of around 200 cc, the amounts excreted were within normal limits

TABLE IV

Comparison of quantitative protein and routine qualitative albumin

Qualitative	Quantitative per cent	Volumes up to 200 cc. mgm
Negative or very faint trace	007	14
Trace to heavy trace	01	20
Heavy trace to +	01 to 05	20 to 100
+ to +++	05 to 1	100 to 200
++ to ++++	1	200

Casts With cast excretion from 6,000 to 79,000 in 12 hours, the qualitative report was "negative' or, in a few instances "1 to 2 per high power field" With counts of 122,000 to one million, most of the qualitative reports were "1 to 2 per H P F" In one case where the count was six million the routine report was "10 to 20 per H P F," while in two other cases where the counts were twelve and fifteen million respectively the routine report was "5 to 10 per H P F" Such discrepancies were not common Four times out of 51 tests in which the cast counts were zero, the routine examination showed a few casts

Red blood cells Among 18 counts which ranged from 222 000 to 2 4 million red cells in 12 hours, there were 11 routine reports of "1 to 10 per H P F" and 7 of "no red blood cells" When the red cell excretion was from 2 5 to 82 million in 12 hours, the routine reports showed "1 to 20 red cells per H P F" As the red cell excretion increased through this range, there was little or no difference in the routine reports With excretions of 189 to 571 million the routine analysis yielded "5 to 60 red cells per H P F" In cases of microscopic hematuria it cannot be said that these methods showed a very close agreement

Epithelial and white blood cells With white cell excretion under one million most of the routine reports were "3 to 5 per H P F' with an occasional ' zero ' report and rarely '10 to 20 per H P F" When the excretion was from 1 to 10 million most of the reports were '5 to 10 per H P F" With excretion from 10 to 75 million the common report was '10 to 20 per H P F," and notes mentioning the presence of 'clumped white cells were frequent

A summary of the comparative results yielded by the two techniques indicates that the major discrepancy occurs in estimating red cells It is likely that this is due in part to the difficulty which even practiced workers experience in recognizing red cells and in part to the fact that books and clinical pathologists have long insisted that the presence of red cells in the urine means some abnormality in the urinary tract The physician, therefore shrinks from reporting erythrocyturia in an individual who has no nephritis papilloma, or renal tuberculosis to account for it In

discussing this matter with laboratory workers, many have admitted that they seldom report an occasional red blood cell in the urine because of its supposedly grave import Traces of albumin and a few white blood cells are commonly reported in well children, and the pediatrician does not become alarmed because of their presence Similarly there is need of a tolerant attitude toward occasional red cells in the urine and such tolerance need not decrease our respect for their significance when they are present in increased numbers

DISCUSSION

The wisdom of introducing any new method of examination which is more time-consuming than the old is questionable unless it can be shown that the character of the information yielded as well as the uses to which it can be put are improved by the new routine The advantages of quantitative data over qualitative data in general do not call for discussion It is essential only to determine whether quantitative urine studies justify in added usefulness the time necessary to procure them A completely satisfactory answer to this question, insofar as it involves the extent to which one method should replace the other, is as yet impossible as the full sphere of usefulness of sediment counts is not yet known At present it would seem that the method should not replace the routine urinalysis Experience, however, has indicated that many cases exist in which the information yielded justifies, from the standpoints of both therapy and prognosis, the added expenditure of time In this clinic the method has proved valuable in studying patients with nephritis, not only in providing quantitative and therefore comparable data for following the course of disease, but also in permitting early recognition of the effect on the kidneys of pharmacologic procedures or intercurrent infection

It has been contended that the method is only approximately accurate The truth of this contention was recognized by Addis (5) who pointed out that it is unnecessary to make the method quantitative in the strict sense of the word "At least for clinical purposes, we want to know the rates in terms of orders of magnitude It is immaterial whether the urine contains one million or one million one hundred thousand casts, although, it is essential to be certain whether there are thousands, tens of thousands, hundreds of thousands or millions" Wide variations are therefore to be expected in normal subjects, and a close division between normal and abnormal is impossible With children the range of variation is further increased by variations in urine volume resulting from inability to enforce rigid fluid restriction With water restricted for 24 hours Addis succeeded in obtaining with adults fairly constant 12 hour night collections of around 380 cc With children especially, therefore, it is essential to hold a rather broad conception of what may come within the range of normal The figures presented in this paper must be regarded as very

roughly approximate only, although the attempt was consciously made to err on the side of including too much, rather than too little, in the normal range

SUMMARY

The technique of Addis for the quantitative determination of the excretion of protein, casts and cells in the urine has been applied to normal children, aged 4 to 12 years The data have been compared with the findings on routine urinalyses and with the results by the Addis technique in adults A tentative standard has been set up for normal children between the ages of 4 and 12 years

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THE ADDIS SEDIMENT COUNT IN SCARLET FEVER[1]

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The following report deals with a study of the urinary changes in ordinary scarlet fever as revealed by the Addis sediment count technique No cases of clinical postscarlatinal nephritis have been included Particular interest centers in the period from 8 to 30 days after the onset of infection

LITERATURE

As early as 1874 Mahomed (1) found that scarlet fever convalescents, at some period during the six weeks after defervescence, exhibited a pulse of high tension (sphygmograph) and hemoglobinuria (guaiac test) without albuminuria (nitric acid test) If the tension was high and persisted albumin was liable to appear Occasionally there was anorexia with slight fever (100° F) and sometimes a little edema was evident upon careful examination, but as a rule, there were no constitutional symptoms or signs He called this picture the "prealbuminuric stage of nephritis "

Thompson (2), in 1886 examined the urine for blood and albumin three times a day for 56 days in 180 cases of scarlet fever Eighty four per cent of the patients were under 15 years In 66 cases (37 per cent) there were no urinary changes Two cases (1 per cent) showed anasarca without urinary changes, and in 112 (62 per cent) the urine showed albumin, blood or both, with or without edema Thompson calls all of these 112 cases "nephritis," but "in some cases, the evidence of kidney mischief was so slight and evanescent that but for careful and frequent testing the presence of these substances, would no doubt, have been overlooked ' Thompson also found at times a positive guaiac test with no albumin, but thought the "pre albuminuric stage ' of nephritis was infrequent

Dittmar (3) examined the urine in 91 consecutive cases twice daily for 56 days Eighty five per cent of the patients were under 15 years The nitric acid test for albumin and the guaiac test for blood were used Forty-eight (52 7 per cent) of the cases at some period showed positive

[1] Two patients were studied through the courtesy of Dr Charles Hendee Smith and 12 patients were studied on the wards of Willard Parker Hospital with the co-operation of Dr George W Caldwell

tests for albumin, or blood, or both He repeatedly found positive guaiac tests with negative albumin

Caiger (4) found traces of albumin in the convalescent stage of scarlet fever occurring in from 30 to 40 per cent of cases He examined the urine only twice a week and noted that in children it was not always possible to do so more regularly

McCrae (5) analyzed 1,034 cases over a period of seven years and found urinary disturbance subsequent to the fall of fever in 25 per cent Five per cent showed definite nephritis, but only 2 per cent presented physical signs and symptoms of nephritis The frequency of testing and methods used are not mentioned

Koch (6) observed a definite increase in blood pressure in every case of scarlet fever from the 14th to the 20th day During this period of hypertension, he found albuminuria, and less frequently, erythrocytes and casts (at times in only a single specimen, and not invariably in every case)

Hirschberg and Ssucharewa (7) have reported more recently on 845 cases of scarlet fever studied over a period of eight years Ninety-two per cent were under 12 years The incidence of nephritis was 20 per cent, but they include a large number (56, or 33 5 per cent of the total) of cases where there were no symptoms and where the urinary changes were slight and did not persist They cite as a typical case in this group a child with no extrarenal symptoms whose urine showed a trace of albumin on the 21st day and an occasional red cell in the sediment for the two subsequent days

Ker (8) stated that it is not unusual to find tube casts in cases of very slight and transient albuminuria The complication tends to occur at the period of convalescence when acute nephritis is most apt to present itself

METHODS AND NORMAL STANDARDS

The technique described by Addis was followed for the counting of casts and cells and that of Shevky and Stafford for the quantitative estimation of protein Methods and minor modifications in the procedure when applied to children have been described in a previous paper (9)

We have tentatively set the following normal standards from a study of 161 counts on 74 normal children aged 4 to 12

The upper limit of normal excretion per 12 hours in children from 4 to 12 years of age

Protein	35 mgm
Casts	10,000
Red blood cells	600,000
Epithelial and white blood cells	600,000 (male) 1,000,000 (female)

OBSERVATIONS IN SCARLET FEVER

Sediment counts were done three times a week for thirty days (in a few cases longer) on 14 cases of scarlet fever The patients selected were all males from 4 to 14 years of age and remained in bed during the entire period on a low protein, salt-poor diet One patient (number 2) had had scarlet fever seven years previously and another (number 1) had had an attack of acute glomerular nephritis following tonsillitis (hemolytic streptococcus) a year earlier None of the patients developed the typical signs and symptoms of postscarlatinal nephritis

The following clinical observations seem pertinent

Temperature In two cases fever persisted to the 9th and 12th days respectively in two others it continued to the 6th day In the rest the temperature was normal by the 4th day or sooner Transient rises in temperature after the first week were observed in three instances

Complications There were no cardiac or arthritic complications Two cases presented a mild cervical adenitis One patient had an unexplained fever on the 23rd day, and another patient developed a bilateral otitis with streptococcus hemolyticus cultured from the discharge

Scarlet fever antitoxin Five patients received from 2,000 to 12,000 units of antitoxin before the 5th day of the disease Serum reactions were observed on the 6th, 7th, 8th, and 9th days following the injections In four of these cases there were slight abnormalities in the sediment count during the serum reaction Analogous changes have been observed in serum sickness following diphtheria and meningococcus antitoxin It is important to note that the patients who received antitoxin and suffered from serum sickness did not show at a later date sediment count abnormalities different from those presented by patients who received no serum

The individual cases are presented graphically in Charts 1, 2, 3, 4 and 5

Urinary studies were not made during the first seven days in two cases, with two exceptions the remaining twelve showed some increase in protein, cast and cell excretion during that period This was interpreted as the ' toxic albuminuria" which occurs during the febrile stage of any acute infection The etiology and pathogenesis of "toxic albuminuria" are not known, yet it seems reasonable to believe that the urinary changes occurring during the febrile period of invasion of an acute infection must have a different origin and interpretation from those which take place in the afebrile period some two or three weeks after the acute onset.

During the period from 8 to 45 days after onset all cases showed in varying degree increases in the excretion of protein, casts and cells The highest levels reached were for protein—174 mgm for casts— 1,320,000, for red blood cells—12,915 000 for epithelial and white blood cells— 9 460,000 The increases were irregular and explosive in character Protein, cast and cell excretion rose and fell together at times but just as

often increased protein excretion was accompanied by normal cast and cell excretion and vice versa As counts were not done daily, we have no accurate information as to the duration of these flare-ups The charts

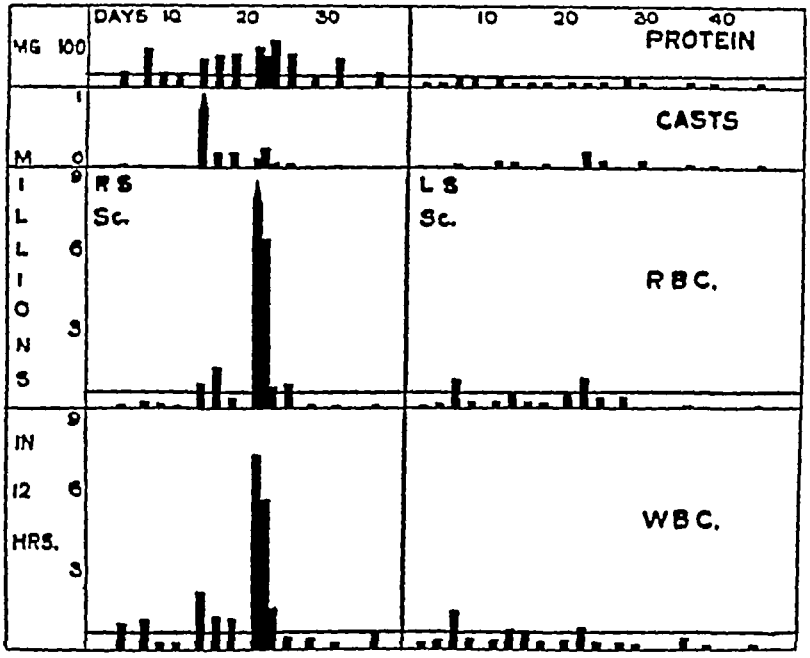

CHART I

R S, 14 years old Scarlet fever mild Temperature normal fourth day, no complications

L S, 6 years old Scarlet fever mild Temperature normal sixth day, no complications Had acute glomerular nephritis one year before

The light lines above the base lines represent the upper normal limits for protein 35 mgm, for red and white blood cells 600,000 Any cast count that appears on the chart is abnormal, i e, above 10,000

of the individual cases show that flare-ups lasting 2 to 4 days were followed by normal or nearly normal counts for 2 to 6 days, when another brief rise occurred followed by a normal period In two cases there were three such rises

There were no serious complications in the group In the four cases that had otitis, unexplained fever or adenitis, the changes during the acute phase of these complications were no more marked than in the uncomplicated cases

DISCUSSION

It is difficult and perhaps not important to draw any definite line between these sub-clinical changes and true postscarlatinal nephritis Except for red cell excretion the higher figures might be found in a mild case of acute nephritis Red cell excretion in an ordinary hospital case of nephritis is usually in the hundreds of millions As Addis (10) points

out, glomerulonephritis is often a symptomless disease at onset. In our experience in children, edema, hypertension and nitrogen retention are not the rule For Addis (11), whose definition of nephritis is based on a

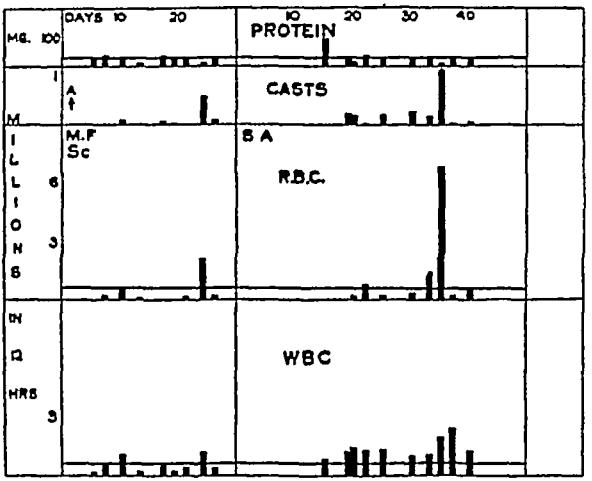

CHART II

M F, 7 years old Moderately severe scarlet fever Scarlet fever antitoxin 15 000 units on the second day, serum sickness on the eleventh day No complications

S A, 5 years old Mild scarlet fever Had adenitis on admission which subsided by the seventeenth day On the thirty sixth day definite increase in casts, red and white cells and temperature 100 5° F for a few hours

The light lines above the base lines represent the upper normal limits for protein 35 mgm for red and white blood cells 600 000 Any cast count that appears on the chart is abnormal i e, above 10,000

quantitative increase above the normal excretion of protein, casts and cells these changes would represent nephritis McCrae (5) notes that in four fifths of his cases of well marked nephritis the diagnosis depended entirely on the urinary examinations Pospischill (12) speaks of an abortive course of nephritis in which only one or a few urinary specimens become hemorrhagic, so that clinically, the nephritis lasts only a few hours Caiger (4) believes that "although changes in the renal tissue are by no means necessarily present in an ordinary attack of scarlatina, yet simple albuminuria of any degree and acute nephritis, when they supervene, are due essentially to the same morbid process varying simply in intensity, or in the vulnerability of the kidney in the particular subject" He supports his view by the following clinical observations.

the relative prevalence of simple albuminuria and acute nephritis in any particular outbreak, their tendency to appear under the same environmental conditions (deficient ventilation, overcrowding), their common

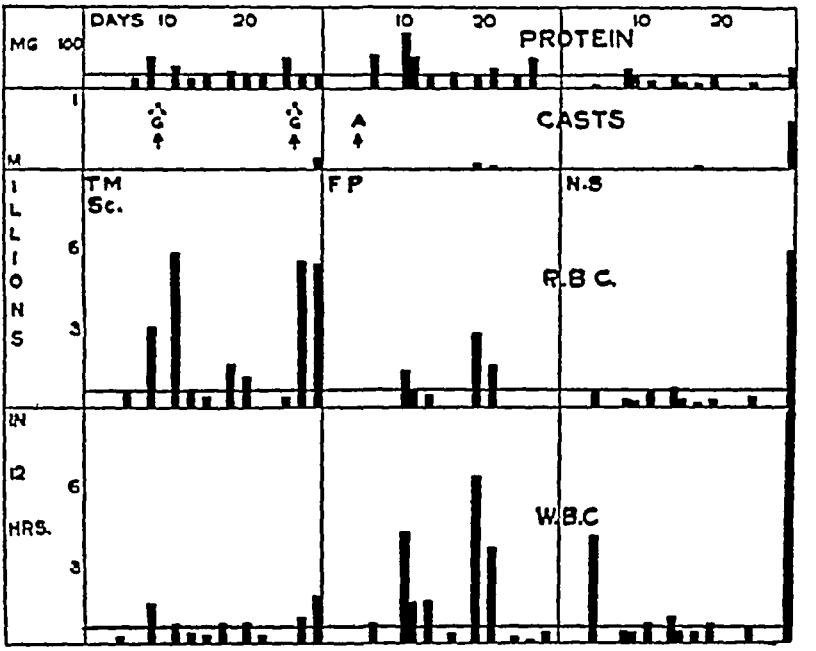

CHART III

T M, 14 years old Mild scarlet fever Seventh to eleventh days cervical adenitis, temperature 100° to 102° F Exacerbation of adenitis twenty-seventh and twenty-eighth days with temperature 100° to 102° F

F P, 14 years old Moderately severe scarlet fever Temperature normal tenth day Scarlet fever antitoxin 10,000 units on the fifth day, serum sickness on the thirteenth day No complications

N S, 4 years old Mild scarlet fever Temperature normal on the fifth day No complications

The light lines above the base lines represent the upper normal limits for protein 35 mgm, for red and white blood cells 600,000 Any cast count that appears on the chart is abnormal, i e, above 10,000

tendency to develop at the same stage of the illness The age liability is also in agreement, that is, the susceptibility to either affection is fairly constant from the second year to the fifteenth Ker (8) says "It is hard to resist the conclusion that the difference between the two conditions (late albuminuria and true nephritis) is only one of degree"

For purposes of discussion we have used the terms "renal irritation" or "micro-nephritis," but until there is a better definition of nephritis, it is immaterial what name is applied The important points are that in 14 cases of scarlet fever there appeared in the urine in slight degree the qualitative changes that are found in nephritis, and that these changes

occurred at the time we expect postscarlatinal nephritis to develop A study of the literature indicates that these changes take place in a great majority of cases of scarlet fever

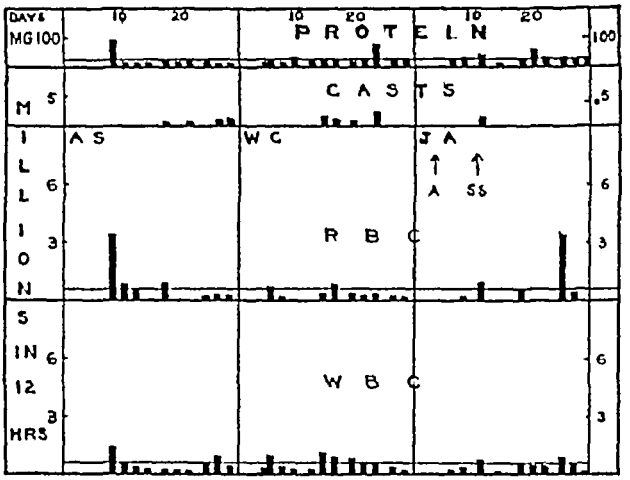

CHART IV

A S, 7 years old Mild scarlet fever, afebrile throughout, no complications

W C, 9 years old Mild scarlet fever, temperature normal 3d day

J A, 7 years old Moderately severe scarlet fever, temperature normal 6th day, 5,000 units scarlet fever antitoxin on 4th day, serum sickness without temperature on 11th day

The light lines above the base lines represent the upper normal limits for protein 35 mgm, for red and white cells 600 000 Any cast count that appears on the chart is abnormal, i e, above 10,000

We have no data to present concerning the mechanism of the urinary changes we are reporting Evidence is accumulating which points to the view that the development of postscarlatinal nephritis is concerned in some way with the immunological reactions of streptococcus hemolyticus Escherich and Schick (13) first drew attention to the analogy between nephritis and the group of allergic reactions and suggested that 'it might be a case of hypersensibility of the organism which is expressed in the ability of small amounts of pathogenic substance to awaken clinical symptoms which at another time would be tolerated without any reaction" More recently this idea has been elaborated by Friedemann and Deicher (14) and by Longcope (15) Since the sub-clinical changes we have observed occur at the same time that true nephritis develops and are qualitatively the same as those found in nephritis it seems likely that the

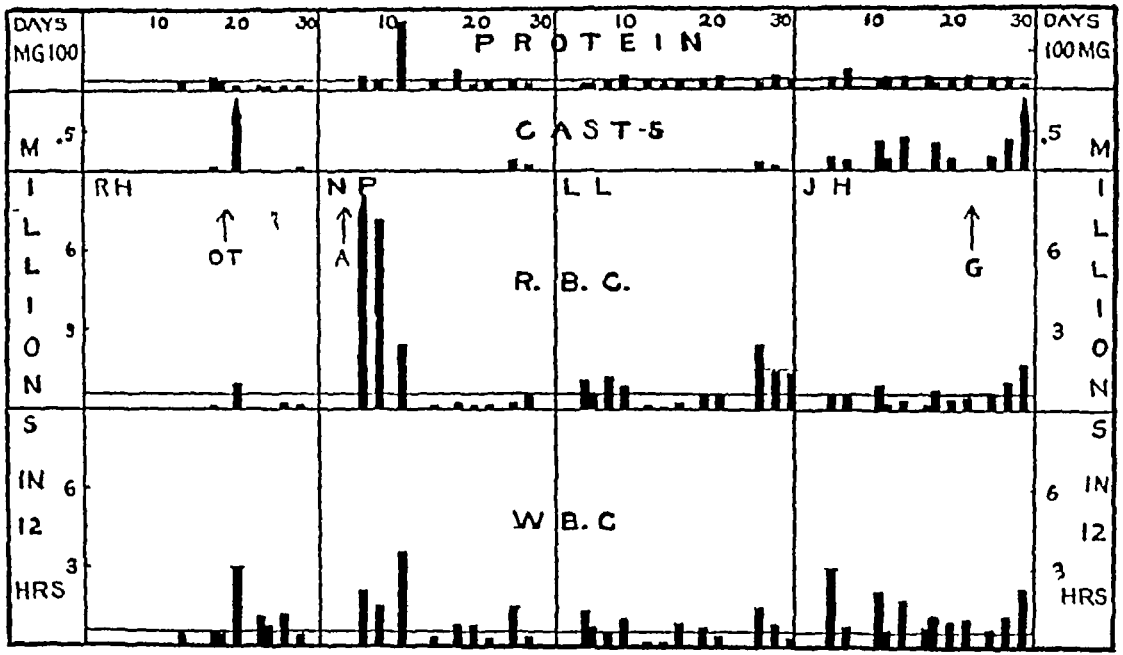

CHART V

A H, 5 years old Moderately severe scarlet fever, temperature to 9th day, 5,000 units scarlet fever antitoxin on 4th day without reaction Otitis media on 18th day temperature 102° F for 36 hours

N P, 13 years old Moderately severe scarlet fever, temperature normal 6th day, 5,000 units scarlet fever antitoxin on 4th day, serum rash on 10th day without fever

L L, 10 years old Mild scarlet fever, temperature 3d day

J H, 9 years old Mild scarlet fever, temperature normal 4th day, cervical adenitis on 22d day without fever

same mechanism is at work in both conditions Our observations and a study of the literature indicate that the majority of individuals who contract scarlet fever make a satisfactory immunological adjustment in the postfebrile period Kidney damage is slight, shown only by unusually careful examination of the urine, the nephritis is aborted A few individuals are unable to make this adjustment and develop true postscarlatinal nephritis

Studies shortly to be published indicate that similar urinary changes occur in children who are infected with streptococcus hemolyticus and streptococcus viridans and in those with an active rheumatic infection, whereas, they occur infrequently in children with pneumococcus infection

SUMMARY

1 The technique of Addis for the quantitative mensuration of protein casts and cells in the urine has been applied to 14 cases of scarlet fever All cases showed moderate transient increases in the excretion of protein and formed elements during the period from 8 to 45 days after onset of scarlet fever

2 The relation of these changes to true postscarlatinal nephritis is discussed

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THE EFFECTS OF TEMPERATURE AND OF TISSUE PRESSURE ON THE MOVEMENT OF FLUID THROUGH THE HUMAN CAPILLARY WALL

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The various physical factors concerned in transporting fluid through the human capillary wall have received relatively little attention in spite of the value that such information might have in clarifying the complicated mechanism of fluid balance and the even more involved mechanism of edema formation The present lack of information is to be ascribed, in all probability, to the difficulty of obtaining quantitative data concerning the filtration and absorption of small amounts of tissue fluid At present the plethysmograph offers the only available means of detecting small changes in the volume of extravascular fluid

The ordinary plethysmograph was found by Krogh, Landis and Turner (1) to be quite unsuitable for measuring the volume of tissue fluid accumulating during short periods of slight or moderate venous congestion Therefore, a so-called "pressure plethysmograph" was devised to exclude spontaneous variations in arm volume referable to changing vasomotor tone Pressure was exerted on the surface of the segment of forearm within the plethysmograph in order to collapse the blood vessels before the final volume was measured Under these conditions the state of contraction or dilatation of the blood vessels did not, within certain limits, interfere with the reasonably accurate measurement of changes in the volume of tissue fluid

It was found in normal subjects that fluid was filtered into the tissues of the forearm when the venous pressure exceeded 15 cm water Above an average venous pressure of 17 cm water the rate of filtration was directly proportional to the increase in venous pressure When the subject stood motionless the colloid osmotic pressure of the blood rose and the rate of filtration produced by given grades of venous congestion fell Thus changes in capillary pressure and in the colloid osmotic pressure of the blood influenced the movement of fluid through the capillary walls of normal human subjects in a manner conforming to the Starling hypothesis

If capillary blood pressure and the colloid osmotic pressure of the blood were the sole factors involved in fluid balance it would be difficult to explain how it is possible for the human being to avoid dependent

edema whenever the erect posture is assumed The observations reported in this paper are concerned with two additional factors which modify conspicuously the rate at which fluid is filtered through the capillary wall It will be shown that the presence of fluid in the tissue spaces diminishes or even abolishes the filtration ordinarily produced by low grades of venous congestion It seems probable, therefore, that fluid accumulating in the tissue spaces develops a tissue pressure which retards filtration and reduces the further loss of fluid from the blood stream

In an effort to diminish the variability in measurements of filtration rates the effects of temperature were studied In extending the studies of Drury and Jones (2) it was found that the rate of filtration depends on the temperature of the forearm even when low venous pressures, well within the physiological range, are used

APPARATUS

The "pressure plethysmograph" used in these observations operated on the same general principles as the one devised by Krogh, Landis and Turner (1) It was modified, however, so that each determination of "reduced arm volume" (i e tissue volume with the blood vessels collapsed) required only two minutes This was accomplished (*a*) by fixing both the plethysmograph and forearm to a heavy iron base, (*b*) by substituting rigid aluminium diaphragms for the rubber ends of the earlier apparatus, and (*c*) by using a pressure of 200 mm Hg (instead of 55 cm water) to compress the segment of forearm within the plethysmograph In addition, by equipping the apparatus with a double wall, the temperature of the plethysmograph and of the water contained within it could be maintained approximately constant throughout each observation

The plethysmograph, shown in Figure 1*A*, was made of brass, 0 5 mm in thickness, shaped in the form of a double-walled truncated cone, having a length of 16 cm The inside diameter at the upper end was 9 cm , at the lower end 11 cm The inner wall (*I*) was separated from the outer (*O*) by a distance of 0 5 cm , making a closed chamber through which water from a 20-liter reservoir was circulated by a small centrifugal pump expelling 6 liters per minute This prevented the water inside the plethysmograph from gaining or losing heat from the air, and kept the apparatus at a relatively constant temperature

A sleeve (*S*) of thin rubber, 0 25 mm in thickness, 36 cm long, and 8 and 10 cm in diameter at the ends, was placed inside the metal case The ends of this rubber sleeve were everted and attached firmly (*S'*) to the ends of the plethysmograph The rubber was cemented to the metal, contact being maintained by stretching a band of rubber, 1 cm wide and 2 mm thick, over the ends of the sleeve and the plethysmograph An adjustable metal hoop, 1 cm in width, encircled each end of the sleeve with its rubber band To avoid complicating the diagram in Figure 1*A*, neither the rubber nor the metal bands are shown The rubber sleeve was made large enough so that when collapsed it extended about 4 cm beyond the ends of the plethysmograph, when filled with water under pressure the sleeve lay against the arm in a series of folds (*F*)

Each end of the plethysmograph was closed by three aluminium diaphragms (*D*), 0 5 mm thick, 14 cm wide and 14 cm long, shaped as shown in Figure 1*B*, which represents an end view of the apparatus The inner edge of each diaphragm was cut out, leaving a semicircular space to accommodate the forearm

With a set of such diaphragms having openings varying between 4 and 10 cm in diameter, forearms of different sizes and shapes could be fitted accurately enough to keep the thin rubber sleeve, even while distended, inside the plethysmograph The diaphragms were held in place by brackets (*B*) attached to each end of the plethysmograph A thick rubber washer (*W*) was placed between the diaphragms and the end of the plethysmograph to prevent the thin rubber sleeve from being cut by the sharp edges of the diaphragms. The central part of this washer consisted of a sheet of thin rubber dam (*DA*) with a central opening having a diameter slightly smaller than the corresponding segment of forearm This prevented the inner bag from escaping through any small space left between the edges of the metal diaphragms and the skin Each diaphragm was slotted (*SL*) so that it could be moved inward or rotated, on the screw (*SC*) until it was in gentle contact with the skin of the forearm The aluminium diaphragms, when thus adjusted, were fastened tightly against the rubber washer and the end of the plethysmograph by screwing a heavy brass ring (*R*), 3 mm in thickness, against the three brackets This prevented the relatively thin aluminium diaphragms from bulging when the pressure inside the plethysmograph was raised

The large opening into the center of the plethysmograph was closed by a rubber stopper (*RU*) which contained a glass tube (*T*) Pressure tubing connected this glass tube with the burette (*BU*) through the double walled glass bulb (*BL*) The space between the inner wall of the plethysmograph (*I*) and the thin rubber sleeve (*S*), the glass bulb (*BL*) the burette (*BU*) and the pressure tubing were all filled with water as shown in Figure 1*C* The temperature of the water in the plethysmograph was measured by means of a thermal junction inserted to the very tip of the glass tube (*G*) which extended about 2 cm beyond the inner surface of the rubber stopper

During each determination of "reduced arm volume' over 100 cc of water moved from the burette (*BU*) into the plethysmograph To prevent the temperature of the water in the plethysmograph from fluctuating, the double walled glass bulb (*BL*) was inserted between the plethysmograph and the burette. The inner chamber of this double-walled bulb had a capacity of 275 cc and communicated with the burette and the inside of the plethysmograph Water from the reservoir and pump passed through the outer chamber of the bulb thence to the space between the inner and outer walls of the plethysmograph and finally back to the reservoir Thus the bulb and the plethysmograph were kept at the same temperature and whenever ' reduced arm volume ' was determined, the water moving from the burette displaced water at a constant temperature from the inner bulb into the plethysmograph

The burette was of the ordinary 100 cc. size with graduations of 0 1 cc A larger tube having a capacity of 100 cc. and a length of 30 cm was fused to the upper end of the burette and calibrated in centimeters The total volume of this modified burette was 200 cc the calibrations were so arranged that changes in total arm volume were read in units of 1 0 cc. (see protocol 1) while reduced arm volumes were read in units of 0 1 cc. The upper end of the burette communicated through a Y tube either with room air or with a 40-liter reservoir containing air under a pressure of 200 mm Hg

Venous pressure was elevated by inflating a pneumatic cuff 50 cm long and 15 cm wide which encircled the arm just above the elbow

PROTOCOL 1

Venous congestion 50 cm water for 10 minutes Subject J G Date April 11, 1932

Time	Burette readings	Temperature: Plethysmograph	Temperature: Arm	Temperature: Air	Notes
min sec	cc	°C	°C	°C	
0 00					Subject recumbent 10 minutes at zero time Plethysmograph adjusted and filled with water at 35° C
					Pressure raised to 220 mm Hg three times to settle rubber sleeve in position
					Top of plethysmograph 18 0 cm above upper surface of arm
					Diameter of arm 8 5 cm
5 00	12 0			18 7	
6 00	11 0		30 6		Pressure raised in plethysmograph, 200 mm Hg
6 30	131 5				
7 00	136 5	34 2			
7 30	137 5				
8 00	138 3				Pressure off
9 00	13 0			19 9	
10 00	40 0		30 6		
11 00	10 0	31 6			
12 00	11 0				
13 00	41 5			19 5	
14 00	11 5		29 1		
15 00	41 0	31 3			
16 00	40 0			20 7	Pressure on in plethysmograph, 200 mm Hg
16 30	136 2				
24 00	40 0				
25 00	39 0			21 9	
26 00	39 5		30 7		Pressure on in plethysmograph, 200 mm Hg
26.30	137 3				
27 00	138 8	31 4			
27 30	139 8				
28 00	140 7				Pressure off
29 00	43 0			21 7	
30 00	40 0		30 7		
31 00	39 0	34 4			
32 00	39 0				
33 00	37 0			21 4	
34 00	37 0		30 9		
35 00	37 0	34 1			
36 00	37 0				Pressure on in plethysmograph, 200 mm Hg
36.30	137 3				
37 00	139 3			22 0	
37.30	140 0				
38 00	140 7		31 5		Pressure in plethysmograph off Venous congestion on, 50 cm water (i e 72 25–22 25 cm)
39 00	23 0				
40 00	18 0				
41 00	15 0			21 1	
42 00	13 0		30 7		
43 00	14 0	34 3			

PROTOCOL 1 (*continued*)

Time	Burette readings	Temperature: Plethysmograph	Temperature: Arm	Temperature: Air	Notes	Time	Burette readings	Temperature: Plethysmograph	Temperature: Arm	Temperature: Air	Notes
min. sec	*cc.*	*° C*	*C*	*C*		*min. sec*	*cc.*	*C*	*C*	*C*	
17·00	139 0		31 0			44·00	12 0				
17·30	139 0					45·00	9 0			21 2	
18·00	139 8	31 2			Pressure off	46·00	9 0		30 0		
19·00	43 0					47·00	8 0	34 4			
20·00	42.0					48·00	6 0				Venous congestion off Pressure in plethysmograph on, 200 mm. Hg
21·00	41 0			22.3		18.30	127 0				
22·00	40.5		30 6			19·00	129.3			22 8	
23·00	40 0	34.2				49.30	131 4				
						50·00	132 6		30.3		Pressure off

Summary

Arm measurements circumferences 27.5 cm
18 0 cm
length of segment in plethysmograph 16.5 cm.
volume 690 cc.

Time *minutes*	Burette reading for reduced arm volume *cc.*
6	138.3
16	139 8
26	140 7
36	110 7
38–48	Venous congestion 50 cm water
48	132.2

Total increase in volume during congestion 8.5 cc.
Rate of filtration 125 cc. per minute per 100 cc of arm

METHOD

The burette-plethysmograph system was first partially filled with water and any air bubbles that had accumulated were removed The pump was started and water at the desired temperature (14 5, 24 5, 34 5 or 44 5° C) was circulated through the outer portions of the plethysmograph and of the glass bulb

In all of the observations described here the subjects were in the recumbent position The arm was abducted and the elbow was flexed at right angles to the upper arm, with the forearm extending vertically The posterior surface of the arm rested on the base of the heavy iron standard as shown in Figure 1*A* In this position the iron base prevented

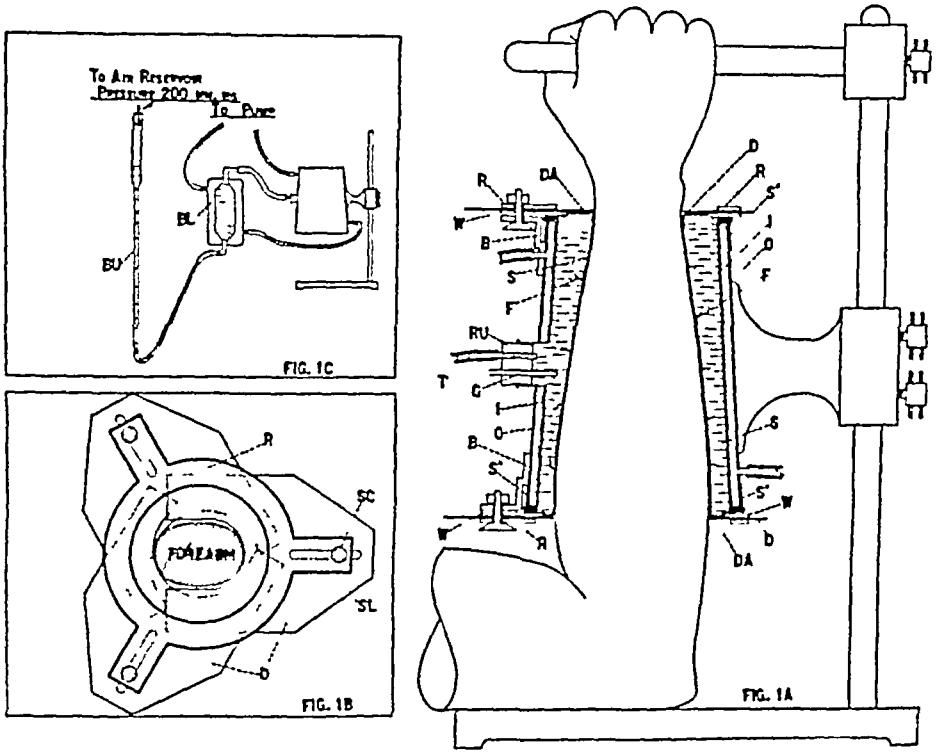

FIG 1*A* DIAGRAM OF THE PRESSURE PLETHYSMOGRAPH, FOREARM, AND STANDARD

For description see text

FIG 1*B* DIAGRAM SHOWING AN END VIEW OF THE PLETHYSMOGRAPH WITH THE THREE DIAPHRAGMS AND HEAVY BRASS RING

FIG 1*C* DIAGRAM SHOWING THE ARRANGEMENT OF THE BURETTE, DOUBLE-WALLED BULB AND THE PLETHYSMOGRAPH

the forearm from moving with reference to the plethysmograph even when the pressure was raised to 200 mm Hg The plethysmograph, with the inner sleeve collapsed, was lowered over the forearm and adjusted so that its midpoint was level with the manubrium sterni The

fingers were lightly flexed over a horizontal bar clamped to the standard and fixed with adhesive tape The aluminium diaphragms were then moved against the forearm and firmly clamped in place.

The position of the arm in relation to the plethysmograph was only relatively secure It was necessary for the subject to remain absolutely still if reliable readings were to be obtained Therefore every effort was made to have the subject entirely comfortable before an experiment began It was usually inadvisable to prolong the duration of an observation beyond two hours because after that time increasing discomfort in the region of the elbow sometimes made the subject restless

With the forearm in position water at the chosen temperature (14 5, 24 5, 34.5 or 44 5° C) was poured into the burette. As the plethysmograph filled, the thin rubber sleeve was pushed against the skin and diaphragms by water displaced from the glass bulb The burette was filled almost completely and its upper end was then connected to the "Y" tube with the limb leading to room air open, and the limb leading to the reservoir containing air at a pressure of 200 mm Hg closed

The surface of the water in the burette was always kept level with the upper edge of the plethysmograph (see Figure 1*C*) The volume of water in the burette plethysmograph system remained unchanged throughout each experiment so that whenever the volume of the arm decreased, the surface of the water in the burette fell below the point of reference—i e the upper end of the plethysmograph The burette was then raised to return the surface of the water to this level and, pressure thus being constant, the volume change could be read directly from the burette When arm volume decreased, the burette reading in cubic centimeters became higher when arm volume increased, the burette reading in centimeters became lower, by an amount equal to the change in arm volume

The term 'reduced arm volume' will be used in the same sense described before by Krogh, Landis and Turner (1), it refers to the volume of the segment of forearm in the plethysmograph when the blood vessels are collapsed by external pressure.

Protocol 1 representing a typical experiment shows the procedure used in measuring the volume of fluid filtered in ten minutes by a venous pressure of 50 cm water The surface temperature of the forearm outside the plethysmograph was measured by means of a thermal junction placed against the skin midway between the lower edge of the plethysmograph and the elbow These readings recorded in Protocol 1 and Table 1 under Arm temperature indicate the skin temperature in those portions of the forearm which were exposed to the air 'Plethysmograph temperature' refers to the water surrounding the segment of forearm within the plethysmograph Air temperature was measured by means of a third thermal junction near the forearm

After the arm had been properly adjusted in the plethysmograph readings of arm volume were begun on the fifth minute, the top of the burette being open to the air On the sixth minute the burette was placed in communication with the 40-liter reservoir containing air at a pressure of 200 mm Hg Water passed rapidly from the burette into the plethysmograph, the burette reading changing from 41 0 to 134 5 cc during the first 30 seconds As water was forced out of the burette into the plethysmograph the burette was slowly raised in its holder so that the surface of the water was always level with the upper edge of the plethysmograph During the first 5 to 10 seconds after pressure was raised the subject experienced throbbing in the forearm but as soon as sufficient water had entered the plethysmograph to elevate the pressure above systolic blood pressure this sensation disappeared The blood expressed from the collapsed blood vessels of the forearm could leave through the open veins proximal to the plethysmograph

The change in volume observed during the first 30 seconds of the pressure period usually amounted to between 80 and 110 cc , the exact figure depending chiefly on the temperature of the forearm Part of this change, about 35 cc , was due to stretching of the rubber tubing, to readjustments of the folds in the rubber sleeve, and to slight bulging of the ends of the plethysmograph During the second period of 30 seconds the change in volume usually amounted to about 2 cc , during the third period of 30 seconds to 1 0 cc and during the fourth period of 30 seconds to between 0 4 and 0 9 cc Only the final reading, obtained after the pressure had been applied for 2 minutes, was used in calculations, but readings were made at intervals of 30 seconds to be certain that the change in volume during compression was quite regular Very occasionally involuntary movements of the muscles of the forearm invalidated a determination of "reduced arm volume," a condition made evident by irregular changes in volume during the period when pressure was applied

After circulation had been occluded for 2 minutes the rubber tube between the compressed air reservoir and the top of the burette was clamped off, the burette being placed once more in communication with room air Water left the plethysmograph and the burette readings changed rapidly to the previous values, varying, in Protocol 1, between 40 and 43 cc After a rest period of 8 minutes (at times only 3 minutes) the determination of "reduced arm volume" was repeated, for comparison with the first reading During the control period this procedure was repeated until the readings of "reduced arm volume" became relatively constant.

It was usual, as shown in Protocol 1, for the second reading of "reduced arm volume" to be lower than the first by 1 0 to 2 0 cc The difference between successive readings became less and less and finally two successive

readings commonly differed by less than 0 9 cc. The control period was prolonged until this was the case in order that the measurement of filtration might not involve large errors due to conspicuous changes in the original "reduced arm volume"

In the experiment shown in Protocol 1 the third and fourth determinations of 'reduced arm volume" were the same At the end of the last control determination (thirty-eighth minute) the pressure in the plethysmograph was released and simultaneously the armlet was inflated to elevate venous pressure in the forearm to 50 cm water

To raise venous pressure in the vertically placed forearm to a given figure, the pressure in the pneumatic cuff encircling the upper arm must be greater by the hydrostatic pressure of the column of blood in the vertical veins of the forearm The segment of forearm within the plethysmograph was supported by water externally so that the effective venous pressure in the forearm enclosed by the plethysmograph was that existing at the upper surface of the plethysmograph Thus to elevate venous pressure in the segment of forearm within the plethysmograph to 50 cm water required that the pressure in the veins of the upper arm be 50 cm water plus the hydrostatic pressure of the column of blood extending from the level of the upper arm veins to the top of the plethysmograph

In the experiment described in Protocol 1 the top of the plethysmograph was 18 cm above the upper surface of the arm The diameter of the arm was 8 5 cm The large veins are situated near the middle of the arm so that the correction was computed by adding to 18 0 cm half the diameter of the upper arm (4 25 cm), making a total correction figure of 22 25 cm Thus to produce a venous pressure of 50 cm water in the forearm required that the pressure in the pneumatic cuff on the upper arm be elevated to 72 25 cm water

This correction figure determined separately for each observation, was always between 22 and 23 cm water The approximate accuracy of this correction was verified further in certain instances by determining the cuff pressure which produced the first measurable engorgement of the veins in the plethysmograph Changes in total arm volume were followed while the armlet was inflated successively to 10, 15 20, 25, 27 5 and 30 cm water pressure It was necessary to elevate the armlet pressure to between 25 and 27 5 cm before engorgement of the veins could be detected in the readings of total arm volume. To measure venous pressure by capsular methods proved to be impossible when the forearm was in the vertical position The need for this correction figure when the forearm is vertically placed, makes it unwise to draw too definite conclusions involving absolute venous pressures It does not affect conclusions based on the comparative effects of various venous pressures or of one venous pressure over long periods of time Hereafter, only the effective venous pressure in the forearm segment will be mentioned, the

corresponding armlet pressure being always greater by between 22 and 23 cm water

The venous pressure used in the experiment recorded in Protocol 1 was 50 cm water, the congestion being continued for 10 minutes The cuff pressure was then released, and three seconds later the pressure in the plethysmograph was again elevated to 200 mm Hg The "reduced arm volume" was 132 2 cc , which, by comparison with the previous reading of 140 7, indicated that tissue volume was 8 5 cc greater as a result of congestion

The apparatus was then removed and the volume of the segment of forearm enclosed by the plethysmograph was determined according to the method previously described (1) The rate of filtration at a given venous pressure was computed by dividing the change in "reduced arm volume" by the duration of the congestion and by the volume of the segment Filtration rate was always expressed in terms of cc of fluid filtered per minute per 100 cc of arm Where volume changes alone were considered they were recorded in terms of cc of fluid per 100 cc of arm

OBSERVATIONS

The studies reported in this paper were made on two male subjects (J G and E L) with normal blood pressures A few experiments were repeated, using other normal subjects, the results were identical with those to be described All filtration rates were determined after the subject had been recumbent for at least 30 minutes

A number of control experiments were made in order to determine the limitations of the apparatus and method It is obvious that the accuracy with which change in volume was measured depended upon (*a*) the uniform distensibility of the apparatus (including rubber tubing, the ends of the apparatus, etc) during repeated periods of elevated pressure, (*b*) the uniformity with which the rubber sleeve applied itself to the surface of the forearm in repeated periods of elevated pressure, and (*c*) the fixation of the arm relative to the plethysmograph

The first two points were tested by determining "reduced arm volume" at intervals of 5 and 10 minutes using a wooden model of the forearm When pressure was raised to 200 mm Hg approximately 35 cc of water left the burette, this change was due chiefly to closer application of the rubber bag to the forearm, stretching of the rubber tubing, and to slight bulging of the diaphragms at the ends of the plethysmograph Repeated readings of "reduced arm volume," each made at the end of the usual two-minute pressure period, were practically constant Thus in one experiment 6 determinations made during 54 minutes were 59 7, 59 8, 59 8, 59 8, 59 8, 59 8 cc In a second experiment 6 determinations made during 51 minutes were 44 0, 44 0, 44 0, 44 0, 44 0 and 44 1 cc These observations made on a wooden model of the forearm, under

conditions otherwise identical with those obtaining during studies on the human subject, show that the elastic parts of the apparatus distended equally in successive determinations and that, with a pressure of 200 mm Hg, the thin rubber sleeve was applied to the surface of the forearm with great accuracy

Nevertheless, whenever a series of readings of "reduced arm volume" were made with the forearm in the plethysmograph the successive values as indicated in Protocol 1 showed a definite tendency to fall off As observed previously (1), this tendency is greater with the first one or two readings becoming less in later readings When the readings were made at intervals of five or ten minutes the first two or three readings usually differed by from 0 8 to 2 0 cc., always indicating an apparent decrease in arm volume After the first three or four readings, however, the decrease in "reduced arm volume' rarely amounted to more, and was usually less than 0 9 cc for a segment of forearm having a volume of approximately 700 cc This slow apparent decrease in "reduced arm volume" continued as long as the determinations were made It was possibly due to the high pressure used, although similar changes in "reduced arm volume' were observed with the much lower pressure used in the earlier method (1) Moreover ' reduced arm volume" decreased slowly when readings were made at intervals of five, ten or twenty minutes Certain comparative experiments described below, indicate that the amount of tissue fluid expressed from the segment of forearm within the plethysmograph by a two minute period at 200 mm Hg must be relatively small

It is also possible (*a*) that placing the forearm in the vertical position may lead to a slow progressive readjustment of the equilibrium between tissue fluid and blood, or (*b*) that the forearm cannot be fixed in position as rigidly as the wooden model In an effort to obtain constant readings in control observations numerous expedients were tried without success In any case the apparent decrease in volume during the latter part of each control period amounted usually to less than 0 015 and rarely to more than 0 020 cc per minute per 100 cc. of arm This change was small in comparison to the filtration rates observed at venous pressures above 20 cm water, and introduced little error in the comparative studies reported here The higher pressure and the vertical position of the forearm could not be avoided since it was essential that readings be made repeatedly at short intervals

By using a high pressure in the plethysmograph the circulation could be stopped in the forearm itself This left the veins of the arm open and the amount of blood which could be conducted away from the compressed forearm was not limited When the armlet was inflated for one minute prior to the determination of reduced arm volume' the amount of blood imprisoned in the veins within the plethysmograph might be as much as 30 to 40 cc Nevertheless, although the determination of ' reduced arm

volume" began even within two or three seconds after the release of congestion the final reading at the end of two minutes' compression varied by only a few tenths of a cc from the control readings made when the "reduced arm volume" was determined with previously undistended veins This indicated that the escape of blood from the forearm was quite complete Additional evidence of the relatively complete removal of blood by the pressure of 200 mm Hg may be found in Figure 5

(*A*) *The relationship between venous pressure and the rate of filtration at 34 to 35° C*

These observations were carried out in two ways In the first series of observations after the subject had been recumbent for at least 30 minutes and after two successive readings of "reduced arm volume" had agreed within 0 9 cc or less, the venous pressure was elevated to 20, 30, 40, 60 or 80 cm water for a period of 30 minutes At the end of the thirtieth minute the venous congestion was released and immediately thereafter the pressure within the plethysmograph was elevated to 200 mm Hg The burette reading at the end of two minutes was compared with the reading taken under similar conditions immediately before the period of congestion The temperature of the plethysmograph was maintained between 34 to 35° C throughout the control and experimental periods

The results of the series of determinations after 30-minute periods of congestion are shown in Figure 2 It may be observed that, in agreement with previous results, when the rate of filtration is plotted against venous pressure the points are distributed about a straight line This line passes through the line of zero filtration at a venous pressure of 14 cm water The value obtained previously (1) was approximately 17 cm water Above 14 cm water the rate of filtration is directly proportional to the venous pressure within the range tested The unit rate of filtration, i e the change in filtration rate corresponding to a change of 1 cm water in venous pressure, amounts to 0 0028 cc per minute per 100 cc The value previously reported (1) for a congestion period of 30 minutes was 0 0023 cc per minute per 100 cc

Similar results were obtained in a series of experiments in which the period of venous congestion was diminished to 10 minutes In Figure 3 are shown the filtration rates observed at a temperature of 34 to 35° C In this series after the usual control period in which two successive readings of "reduced arm volume" agreed to within 0 9 cc or less, venous pressure was elevated to between 5 and 60 cm water The congestion was continued for only 10 minutes and then released, to be followed immediately by another determination of "reduced arm volume" Under these circumstances the spread of the filtration rates observed at any given venous pressure was considerably greater This was to be

expected because of the greater error involved in measuring the smaller total amounts of fluid The general relationship between venous pressure and rate of filtration is, however, the same in that the points are dis

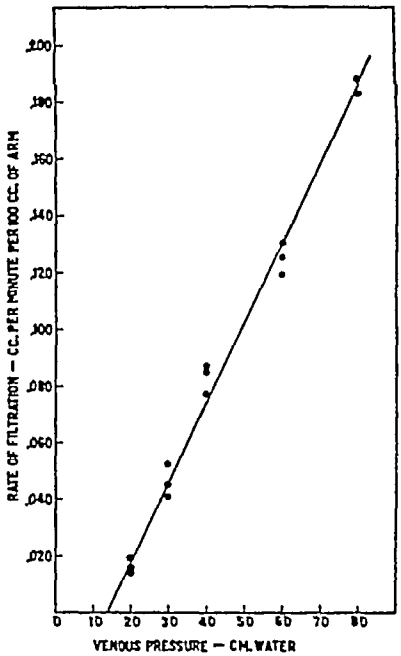

FIG 2 SHOWING RATES OF FILTRATION PRODUCED DURING 30 MINUTES BY VENOUS PRESSURES BETWEEN 20 AND 80 CM WATER
Plethysmograph temperature was 34 to 35° C

tributed about a straight line This line intersects the line of zero filtration at 10 cm water Above 10 cm water the rate of filtration is directly proportional to venous pressure The rates of filtration obtained during 10 and 30 minute periods differ in two respects In the first place the unit change in rate of filtration with the ten minute periods is somewhat greater than that observed with the thirty minute periods of congestion, amounting with the shorter period to 0 0033 cc. per minute per 100 cc. of arm for a change of 1 cm water in venous pressure In the second place, at any given venous pressure the average rate of filtration in cc. per minute per 100 cc of arm during 10 minutes is greater than that observed during thirty minutes

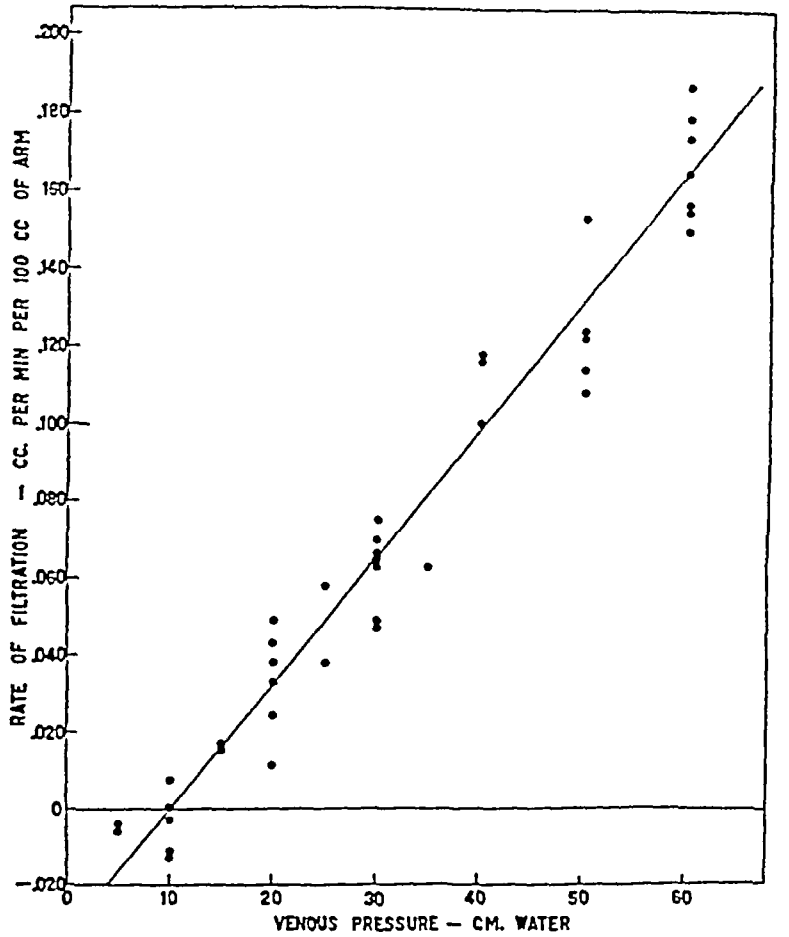

FIG 3 SHOWING RATES OF FILTRATION PRODUCED DURING 10 MINUTES BY VENOUS PRESSURES BETWEEN 5 AND 60 CM WATER

Plethysmograph temperature was 34 to 35° C

(*B*) *The relation between local temperature and the rate of filtration*

Drury and Jones (2) have already shown that at high venous pressures temperature conspicuously modifies the rate of filtration in the lower extremities It seemed essential to determine whether a similar effect was to be observed at lower venous pressures more nearly within the physiological range Figure 4 shows the change in "reduced arm volume" observed after ten minutes of congestion at 60 cm water with plethysmograph temperatures at 14 to 15, 24 to 25, 34 to 35, and 44 to 45° C Each symbol (dot, circle, etc) represents a determination of "reduced arm volume," requiring two minutes' stoppage of circulation During the control period it may be observed that "reduced arm volume" diminished slightly A venous pressure of 10 cm water failed, except at the lowest temperature, to produce any significant filtration At 14 to

15° C a venous pressure of 60 cm water filtered during 10 minutes approximately 0 9 cc. of fluid per 100 cc of tissue, or 6 3 cc. in a segment of forearm with a volume of 700 cc At 24 to 25° C , 34 to 35° C and 44 to 45° C the amount of filtered fluid became greater with each step in

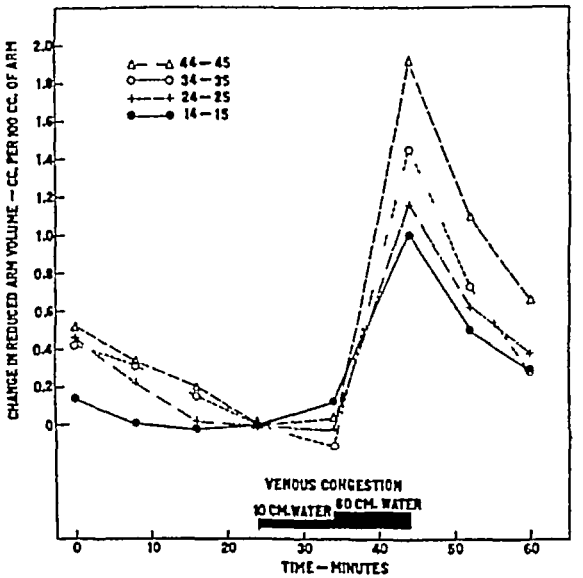

FIG 4 SHOWING CHANGE IN ' REDUCED ARM VOLUME" PRODUCED BY ELEVATING VENOUS PRESSURE TO 10 CM WATER FOR TEN MINUTES, AND TO 60 CM WATER FOR TEN MINUTES, AT TEMPERATURES OF 14 TO 15 24 TO 25, 34 TO 35 AND 44 TO 45° C

Each symbol (circle dot etc.) represents a determination of "reduced arm volume requiring two minutes complete stoppage of blood flow

temperature The rate of filtration at 44 to 45° C was approximately twice that observed at 14 to 15° C

Table 1 shows the results of a larger series of observations made at these four temperatures using venous pressures from 10 to 60 cm water With a venous pressure of 10 cm water slight filtration was observed with a temperature of 14 to 15° C but no filtration could be detected at the higher temperatures All the figures however, are within the range of error of the method With a venous pressure of 20 cm water filtration was observed at all temperatures but the average rates did not differ significantly, the average figures varying from 031 cc.

TABLE 1

Effect of plethysmograph temperature on filtration rate

Venous pressure	11–15° C				24–25° C				31–35° C				41–45° C			
	Temperature			Filtration rate	Temperature			Filtration rate	Temperature			Filtration rate	Temperature			Filtration rate
	Air	Arm*	Plethysmograph		Air	Arm*	Plethysmograph		Air	Arm*	Plethysmograph		Air	Arm*	Plethysmograph	
cm water	°C	°C	°C	cc per minute per 100 cc	°C	°C	°C	cc per minute per 100 cc	°C	°C	°C	cc per minute per 100 cc	°C	°C	°C	cc per minute per 100 cc
10	21 6	29 0	11 1	012	23 6	30 7	21 5	− 001	21 3	34 7	31 8	− 013	25 6	36 6	41 3	− 003
	25 8	26 6	11 0	000	22 0	32 1	21 5	− 007	21 7	33 7	31 3	007	21 9	37 1	11 6	003
	21 1	26 2	11 1	010	23 3	32 6	21 7	008	23 7	33 8	31 5	− 011	25 3	38 0	11 9	006
				007				− 001	23 3	35 3	31 0	000	25 3	35 5	15 5	− 005
									25 7	35 2	34 1	− 003	26 8	36 5	13 8	− 011
												− 001	28 0	36 3	11 2	− 008
																− 003
20	23 8	28 9	11 3	030	22 1	30 1	21 1	031	24 7	33 5	31 2	024	22 0	35 0	12 9	035
	25 6	29 6	15 3	031	23 6	29 6	24 5	037	21 1	33 7	31 6	011	22 5	35 0	42 0	048
				031	21 8	30 9	21 8	019	25 7	32 8	31 8	026				042
					23 2	31 3	21 5	050	26 0	34 7	31 8	038				
					25 7	31 3	21 6	043	27 1	34 8	34 9	043				
					21 0	29 0	21 8	024	24 1	34 3	31 6	033				
								040	25 5	33 7	34 5	049				
												032				
30	25 6	25 6	14 2	042	22 4	26 9	21 3	055	25 3	34 5	34 0	067	25 8	36 8	44 2	073
	21 1	24 0	14 5	045	22 6	30 9	24 5	039	24 9	31 6	34 4	060	27 9	36 5	14 3	073
				044				047	22 9	31 4	34 2	047				073
									21 0	33 5	34 3	070				
									25 0	33 6	34 5	049				
									23 4	34 0	34 5	075				
												061				

TABLE 1 (*continued*)

Venous pressure	14–15° C.					24–25 C.					34–35° C					44–45 C.				
	Temperature			Filtration rate		Temperature			Filtration rate		Temperature			Filtration rate		Temperature			Filtration rate	
	Air	Arm	Plethysmograph			Air	Arm	Plethysmograph			Air	Arm*	Plethysmograph			Air	Arm*	Plethysmograph		
cm water	*C*	*C*	*C*	*cc per minute per 100 cc*		*C*	*C*	*C*	*cc per minute per 100 cc*		*C*	*C*	*°C*	*cc per minute per 100 cc*		*C*	*C*	*°C*	*cc per minute per 100 cc.*	
40	25 9	26 4	14 5	052	062	23 2	29 2	24 8	075	070	23 4	34 5	33 9	118	112	24.5	34 1	44 0	128	125
	25 9	29 2	14 9	062		25 7	30 7	24 6	068		25 4	34.3	33 8	117		24 1	35 2	44 0	121	
	22 6	28 0	14 2	073		25 7	30 6	24 6	068		21 6	33 9	34 5	101						
50	23 4	28 0	14.8	076	082	24 6	28 8	24 7	086	089	21 6	30 7	34 4	124	128	25 3	38 7	45 0	168	169
	24.8	29 2	14 6	108		23 0	30 0	24 7	091		23 9	33 8	34 8	153		26 0	35 7	45 0	169	
	22 7	27.8	14.2	061							20 9	33 4	34 6	122						
											24 0	33 4	35 0	114						
60	25 2	27 9	14.3	089	110	23 5	29 9	24 3	118	115	24 5	33.5	34 8	165	167	25 9	36 6	44.3	221	205
	24 0	25 7	14 5	131		22 6	31 5	24 7	112		23 6	33 2	34.5	156		25 0	37 4	44 8	188	
											23 5	33 9	34 6	156						
											25.3	33 7	34 7	179						
											23 1	33 0	34.5	174						
											23 4	33 4	34 5	166						
											20 5	32 6	34 4	188						
											27 3	34 8	35 0	148						

* Arm temperature refers to the skin temperature of the forearm outside the plethysmograph

"reduced arm volume" rose at approximately the same rate as did total arm volume

In both curves of "reduced arm volume" the rate of change is most rapid during the first few minutes becoming gradually slower as the period of congestion lengthens Thus, during the first 6 minutes a venous pressure of 60 cm water filtered fluid at a rate of 1 0 cc per minute, whereas during the last ten minutes of a 75 minute period the same venous pressure filtered fluid at a rate of only 25 cc per minute

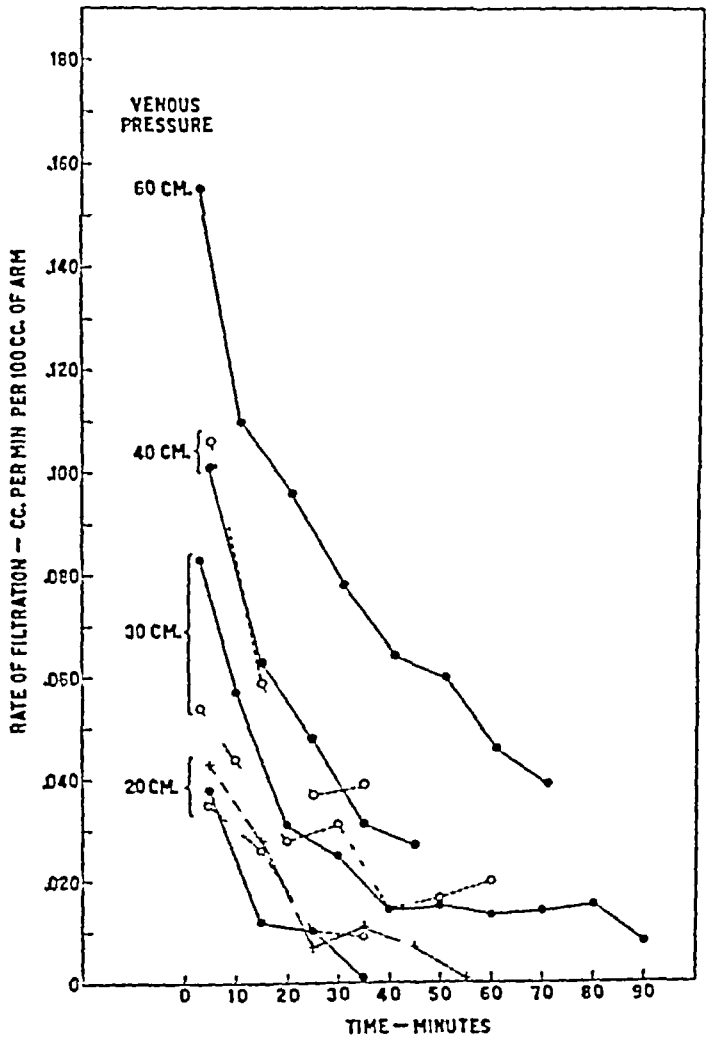

FIG 6 SHOWING THE CHANGE IN THE RATE OF FILTRATION WHEN VENOUS PRESSURE WAS ELEVATED FOR LONG PERIODS OF TIME

The gradual decrease in filtration rate when venous pressure was elevated over a long period of time is shown in Figure 6 In this series of

observations venous pressure was elevated to 20, 30, 40 or 60 cm water for periods totalling between 35 to 90 minutes, while "reduced arm volumes" were determined repeatedly usually at intervals of 10 minutes The rate of filtration produced by any given venous pressure decreased conspicuously as the period of congestion lengthened Thus a venous pressure of 60 cm water filtered fluid into the tissue spaces at the rate of 156 cc. per minute per 100 cc. of arm during the first 6 minutes The rate was reduced to 110 between the 7th and 15th minutes, and to 040 between the 65th and 75th minutes

Similar results were obtained with a venous pressure of 40 cm water Filtration rates originally slightly above 100 cc per minute per 100 cc. of arm were reduced after 35 or 40 minutes to less than 040 cc per minute per 100 cc of arm A venous pressure of 30 cm water filtered fluid at rates of 054 and 085 cc per minute per 100 cc of arm during the first 5 minutes and at rates of 020 and 013 cc per minute per 100 cc. of arm at the end of 60 minutes A venous pressure of 20 cm water filtered fluid at a rate of about 040 cc. per minute per 100 cc. of arm during the first 10 minutes of congestion In two experiments no filtration could be detected after 35 and 55 minutes of congestion respectively From this it appears that a venous pressure of 20 cm water may after a time completely lose its power to produce filtration A venous pressure of 30 cm water produced filtration even after a period of 90 minutes

From the preceding results it is obvious that the effectiveness of any given venous pressure in producing filtration depended, among other things, upon the length of time during which the venous pressure had been acting The longer the period of congestion, the greater was the volume of fluid in the tissue spaces and the slower the rate of filtration If this change in filtration rate is due to the accumulation of fluid the filtration rate observed should depend upon the amount of fluid in the tissue spaces and not upon the venous pressure which caused it to accumulate there.

This was tested by measuring filtration during alternating periods of congestion with pressures of 20 (or 40) cm water and 60 cm water, as shown in Figure 7 After a series of control measurements of "reduced arm volume" venous pressure was elevated for a period of ten minutes to 60 cm water The second determination of "reduced arm volume" was followed in one experiment by a ten minute period at 40 cm and in another by a similar period at 20 cm This alternation of high and low venous pressures was continued until fluid was no longer filtered at the lower pressures

When venous pressure was raised to 60 cm water during a series of short periods the "reduced arm volume" increased more and more slowly during each succeeding ten minute period (Figure 7, dots and solid lines) When venous pressure was raised alternately to 60 cm. and 40

cm water the latter pressure produced slight filtration on the first occasion, negligible filtration on the second, and on the third and fourth occasions fluid was slowly removed (Figure 7, circles and dotted lines) Hence, elevating venous pressure to 60 cm water for a sufficiently long period of time completely prevented subsequent filtration at 40 cm water Apparently an accumulation of 4 cc of fluid per 100 cc of tissue was sufficient to abolish the further filtration of fluid by a venous pressure of 40 cm water

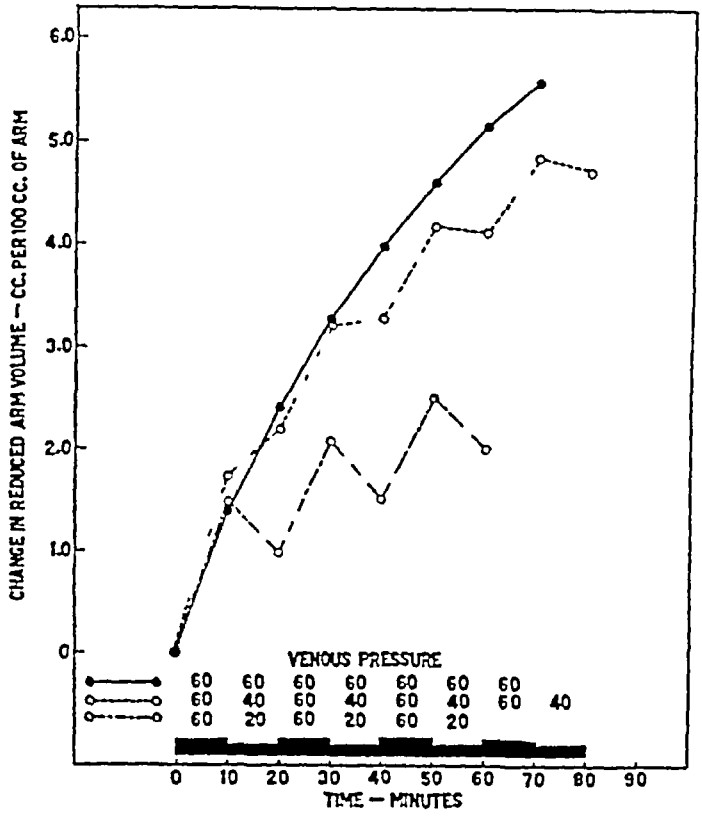

FIG 7 SHOWING CHANGE IN "REDUCED ARM VOLUME" (*a*) DURING A SERIES OF PERIODS WITH A VENOUS PRESSURE OF 60 CM WATER, (*b*) DURING A SERIES OF PERIODS WITH VENOUS PRESSURES OF 60 AND 40 CM WATER ALTERNATELY, AND (*c*) DURING A SERIES OF PERIODS WITH VENOUS PRESSURES OF 60 AND 20 CM WATER ALTERNATELY

In previous observations a venous pressure of 20 cm water always produced slight but definite filtration into a forearm containing only the normal amount of tissue fluid A single ten-minute period of venous congestion at 60 cm water pressure was sufficient, however, to abolish the filtration usually produced by 20 cm water The accumulation of 1 5 cc of fluid per 100 cc of arm resulted in the rapid removal of fluid even with a venous pressure of 20 cm water

The decrease in filtration rate observed at various venous pressures from 20 to 60 cm water has been charted in Figure 8 against the volume of tissue fluid in the arm at the beginning of the congestion period Each point represents the filtration rate observed during a period of ten minutes The amount of fluid in the tissues of the forearm at the beginning of this period was computed from known increase in "reduced

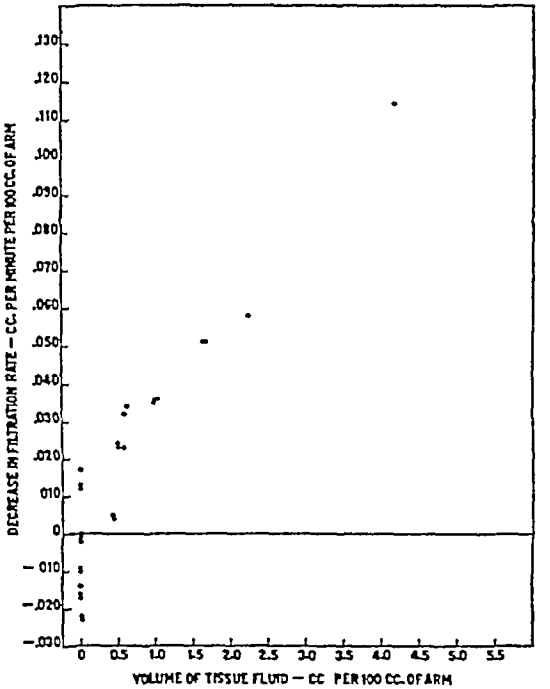

FIG 8 SHOWING THE EFFECT OF ACCUMULATIONS OF TISSUE FLUID ON FILTRATION RATE

For full description see text.

arm volume" produced by previous congestion The filtration rate observed at venous pressures of 20, 40 or 60 cm water with a known volume of fluid in the tissue spaces was subtracted from the average filtration rate observed (Figure 3) at the same venous pressure when no fluid had been previously filtered into the arm The difference between these two filtration rates was charted against the volume of tissue fluid present at the beginning of the congestion period

It may be observed from Figure 8 that the greater the volume of tissue fluid the more conspicuous was the decrease in filtration rate The points arrange themselves roughly about a curve which approaches a straight line in its left hand portion When the excess tissue fluid amounted to 5 0 cc per 100 cc of forearm the filtration rates were on the average 110 cc per minute per 100 cc of arm less than the filtration rates observed at the same venous pressures in a forearm free of previously filtered fluid

From previous results (Figure 3) it was computed that when filtration was measured in 10-minute periods a change in venous pressure of 1 cm water modified the filtration rate by 0033 cc per minute per 100 cc of arm A change in filtration rate of 110 corresponds to a change in venous pressure of approximately 35 cm water Thus, in the presence of an accumulation of tissue fluid amounting to 5 0 cc per 100 cc of arm a venous pressure of 60 cm water filters fluid at a rate comparable to that produced by a venous pressure of 25 cm water in a forearm containing only the normal amount of tissue fluid It would be expected that if 5 0 cc of fluid per 100 cc of arm were already present, a venous pressure of 20 or 40 cm water would be unable to produce further filtration This was actually the case, as shown in Figure 7

It might possibly be argued that these findings were artefacts produced by the method of measuring "reduced arm volume" When venous pressure is elevated for a prolonged period the amount of tissue fluid increases If the application of a pressure of 200 mm Hg to the forearm squeezed large amounts of fluid from the tissues inside the plethysmograph into the tissues just outside, the amount of fluid thus lost would probably become greater as the amount of tissue fluid increased Under these conditions, the decrease in filtration rate might be regarded as due to purely mechanical errors introduced by the method That this is definitely not the case is shown in Table 2 In this series of observations after the usual control readings had been made venous pressures of 20, 30, 40 or 60 cm water were used (*a*) in a series of short periods, one following immediately after the other, and (*b*) in one long period having a duration equal to the sum of the short periods Now, during a total filtration period of 30 minutes when determinations were made after each of three consecutive ten-minute periods of congestion, the arm was compressed three times while with a single long filtration period of like duration the arm was compressed only once If there were any significant decrease of "reduced arm volume" directly due to the external application of pressure it would be expected that the observation including three determinations would show a much smaller change in volume than the observation requiring but one determination

TABLE 2

A comparison of the amount of fluid filtered at various venous pressures during several successive short periods and during a single long period

Venous pressure		Successive short periods		Single long period		Venous pressure		Successive short periods		Single long period	
		Duration	Fluid filtered	Duration	Fluid filtered			Duration	Fluid filtered	Duration	Fluid filtered
cm. water		minutes	cc. per 100 cc. of arm	minutes	cc. per 100 cc. of arm	cm. water		minutes	cc. per 100 cc. of arm	minutes	cc. per 100 cc. of arm
		10*	.33					5	42		
20		10	26	30	48			10	57		
		10	10			30		10	.31	35	1.58
	Total	30	69					10	25		
		10	38				Total	35	1.55		
20		10	12	30	58			10	1 02		
		10*	10			40		10	63	30	2.62
	Total	30	60					10	48		
		10	43				Total	30	2 13		
20		10*	28	30	43			10	1 06		
		10*	07			40		10	.59	30	2.31
	Total	30	78					10	.37		
		5*	45				Total	30	2 02	30	2.54
		10	62					6	93		
30		10*	45	35	1 87			9	99		
		10*	34			60		10	96	35	4 15
	Total	35	1 86					10	78		
		5	27				Total	35	3 66		
		10	44					10	1 79		
30		10	28	35	1 43	60		10	86	30	3 75
		10	31					10	82		
	Total	35	1.30				Total	30	3 47	30	3 90

* Venous congestion continued during readings of ' reduced arm volume.

Examination of the figures in Table 2 will show that this was not the case Thus in three experiments with a venous pressure of 20 cm water the amount of fluid filtered in the single long period was not greater than the amount of fluid filtered in three short periods of like total duration The same was true with a venous pressure of 30 cm water For example in 35 minutes a venous pressure of 30 cm water applied continuously filtered 1 87, 1 43 and 1 58 cc. of fluid per 100 cc of arm. The same venous pressure, applied for one period of five minutes and three of ten minutes each, filtered total volumes of fluid amounting to 1 86, 1.30 and 1 55 cc The difference between these two sets of figures is not significant At venous pressures of 40 and 60 cm water the totals differed by not more than 0 52 cc per 100 cc of arm indicating that the amount lost with each single determination of reduced arm volume is, at most 0 25 cc per 100 cc. of arm for each two minute period of 200 mm

Hg pressure This is much too small to explain the decreasing filtration in prolonged periods of congestion This, we believe, is due to mounting tissue pressure in the intercellular spaces distended by the filtered fluid

(D) The rate at which tissue fluid is removed

The volume of tissue fluid present in the forearm exerts a significant effect on the rate at which that fluid is removed The method of determining the rate of removal of fluid consisted in repeating the observations of "reduced arm volume" at intervals of 10 minutes after the end of one or more periods of congestion When venous pressure was not elevated the rate at which fluid was removed from the tissue spaces depended primarily upon the amount of fluid present When a large amount of tissue fluid was present the "reduced arm volume" returned to normal very much more rapidly than when a small amount of tissue fluid was present

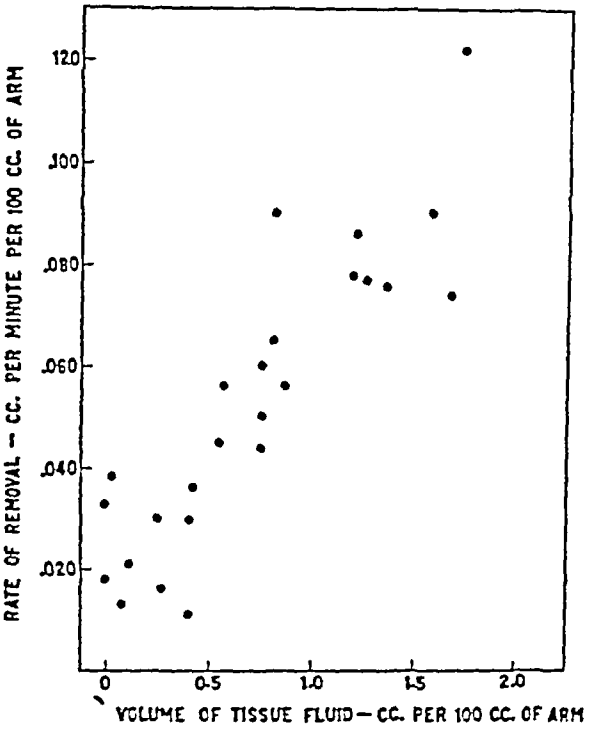

FIG 9 SHOWING THE RELATION BETWEEN THE VOLUME OF TISSUE FLUID AND THE RATE OF REMOVAL OF THAT FLUID
Plethysmograph temperature was 34 to 35° C

The change in "reduced arm volume" during the removal of fluid is shown in Figure 4 In these observations at the end of the filtration period the armlet was deflated and two or more readings of "reduced arm volume" were made at intervals of ten minutes The relation between

the volume of tissue fluid and the rate of removal is shown by Figure 9 which includes only observations made at a temperature of 34 to 35° C The points are distributed roughly about a straight line. The scattering, however, is so great that it hardly seems justifiable to draw any conclusions concerning the slope and position of that line. It is clear, however, that the rate at which fluid leaves the forearm is related to the amount of tissue fluid which has accumulated during the preceding period or periods of filtration

The effect of temperature on the removal of fluid is not striking except possibly at 14 to 15° C Inspection of Figure 10 will show that the points

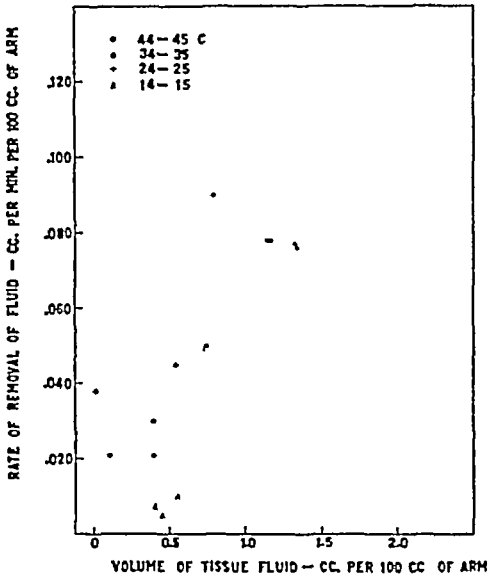

FIG 10 SHOWING THE EFFECT OF TEMPERATURE ON THE RATE AT WHICH TISSUE FLUID WAS REMOVED WHEN VARYING VOLUMES OF TISSUE FLUID WERE PRESENT

representing the removal of fluid at 44 to 45, 34 to 35, and 24 to 25° C are distributed over approximately similar areas However, when the plethysmograph temperature was dropped to 14 to 15° C small amounts of tissue fluid sometimes failed to leave the tissues as rapidly as similar amounts when the forearm was at a higher temperature On the other hand when large amounts of fluid had accumulated the rate of removal was only slightly, if at all, slower at the lower temperature.

DISCUSSION

There is evidence from several sources that temperature modifies the balance between tissue fluid and the fluids of the circulating blood Bazett (4) has called attention to the common knowledge that the limbs swell during warm weather and shrink during cold weather This change in volume may be due to some extent to the amount of blood in the tissues, though the rate of change is too slow to be explained satisfactorily on this basis It is a matter of common observation also that rings, gloves, and shoes appear to be tighter in summer than in winter Experimentally Okuneff (5) has shown that dyes pass more rapidly into areas of tissue which have been heated Moreover, dyes are removed more rapidly by way of the lymphatic vessels from such heated areas Drury and Jones (2) immersed the legs and feet in baths ranging in temperature from 14 to 42° C and found, with the ordinary plethysmograph, that the filtration produced by venous pressures of 40 to 80 mm Hg was very much greater at the higher temperatures

Indirect measurements by Lewis and Haynal (6) and direct determinations by Landis (7) indicate that warming the skin elevates cutaneous capillary pressure conspicuously Moreover, local heat, by dilating capillaries previously closed or only partially open, must increase the total filtering area quite considerably Both factors, or either factor singly, would tend to increase the filtration rate at any given venous pressure It is impossible to estimate the relative importance of these two possibilities from the data at present available

Hamilton and Barbour (8) studied the effects of cold on the transport of fluid from the blood to the tissue spaces When anesthetized dogs were kept lying on slabs of ice for 20 to 50 minutes immediately before they were killed, it was possible to demonstrate a larger content of water in the subcutaneous tissue and muscle of the cooled side Barbour and Hamilton (9) postulated, therefore, that exposure to cold produces anhydremia because fluid is diverted to the cooled skin, subcutaneous tissues and muscles as a result of arteriolar constriction, capillary dilatation from anoxemia, and increased permeability In the plethysmographic studies here described lowering the temperature of the forearm to 14 5° C did not cause the "reduced arm volume" to increase As Bazett (4) points out, the generalizations made by Barbour and Hamilton seem unwarranted at present for no control observations were made to determine the effect of gravity Moreover, an ice block might well be an excessive stimulus, giving results comparable with slight frost-bite rather than with those of ordinary cold Lewis (10) has postulated that the reactive vasodilatation which comes on when the skin is cooled below 15° C is due to an axone reflex, stimulated by injury and the release of H-substance in the cooled skin

In studies of active muscle and secreting glands Barcroft and Kato (11) recorded rates of lymph flow in cc per gram of tissue per minute. These figures, obtained indirectly by comparing hemoglobin contents of arterial and venous blood, are considerably greater than the highest rates of filtration found in the forearm during simple congestion The rapid flow of lymph from active tissues may be partly due to the extravascular accumulation of metabolites suggested by Asher and Barbèra (12)

The striking effect of temperature on the filtration rate at venous pressures of 30 or more cm water is of particular interest with regard to the appearance of edema, particularly of those types related to climatic changes Frequently patients with mild edema complain that the swelling of the lower extremities is greater during warm weather than during cold Castellani (13), in a recent review of minor tropical diseases, mentions two types of heat edema The milder form is "extremely common all over the tropics, and Europeans on their way to the East frequently develop it as soon as the boat reaches the southern portion of the Red Sea and Aden " The feet and legs swell slightly and pitting may occasionally occur, without any evidence of renal or cardiac abnormality A more severe form, "heat oedema gravis," was observed by Castellani in Europe and America during severe heat waves The edema comes on suddenly, lasts as long as the high temperature persists and then disappears This type of edema involves for the most part, the lower extremities, pits slightly on pressure and, like the first variety, is unaccompanied by any abnormalities of renal or cardiac function

In a warm environment, as shown by Lewis and Pickering (14), the cutaneous vessels of the upper extremities dilate in order to promote loss of heat Gibbon and Landis (15) found that immersing the forearms in warm water induces in the lower extremities a maximal vasodilatation which is comparable in degree to that obtained by spinal anesthesia Under these conditions the total area of capillary wall available for filtration in the extremities must be much increased In the erect posture venous pressure in the lower extremities may reach very high levels due to the hydrostatic pressure of the column of venous blood In the presence of such high venous pressure, an increase in the capillary area available for filtration would favor the accumulation of greater amounts of fluid in the tissue spaces Heat edemas may, therefore, be at least partially due to physical effects similar to those exerted by local heat on the filtration of fluid through the normal capillary wall

Observations on the filtration produced by continued venous congestion show that beginning with the first minutes of congestion, some force diminishes the power of a given venous pressure to filter fluid from the blood into the tissue spaces This might be due (*a*) to a change in the membrane in the direction of decreased permeability or (*b*) to some force in the tissue spaces opposing capillary pressure As evidence that the

first possibility cannot explain the findings it may be mentioned that when venous pressure was elevated for two periods of ten minutes each, the rate of filtration during the second period was always less than that during the first Then if the fluid was allowed to leave the forearm and the determination was repeated, using the same venous pressure, the filtration during the third period of ten minutes was approximately the same as that observed during the first period Moreover, the decrease in filtration rate depended quite definitely (Figure 8) on the amount of fluid in the tissue spaces

Krogh, Landis and Turner (1) observed that an accumulation of fluid in the tissue spaces diminished or even abolished the power of venous pressures of 15 to 30 cm water to produce filtration Lymphatic drainage and tissue pressure were both considered in the discussion of these results but the limitations of the earlier method precluded any definite decision concerning the relative importance of these two factors

From the observations reported in this paper it appears that tissue pressure is the more important Filtration rates measured during the first period of congestion were uniformly higher than those in succeeding periods even when the armlet was inflated continuously during the time when "reduced arm volume" was measured (Table 2, first four experiments) Under these conditions lymph could leave the arm only if pressure in the lymphatic vessels exceeded the pressure in the armlet The change in "reduced arm volume" produced by prolonged periods of venous congestion amounting to 20 or 30 cm water was not significantly changed by thus preventing the complete emptying of the lymphatics during each determination of "reduced arm volume"

Drury and Jones (2) found that at a given venous pressure the rate of filtration between the tenth and twentieth minutes of congestion was greater than that between the twentieth and thirtieth minutes They were, however, unable to measure the volume of fluid filtered during the first ten minutes of congestion, this explains in part the relatively low filtration rates reported in their paper

The rates of filtration observed in the forearm during the first ten minutes of venous congestion were higher than those observed during the first 30 minutes of venous congestion (Figures 2 and 3) This difference must be related to the rapid decrease in the rate of filtration observed during long continued venous congestion (Figure 6) The observations are in accord with the conception that, as fluid accumulates in the tissue spaces, an increasing pressure is required to overcome the elasticity of the separated tissue elements This tissue pressure becomes greater as the volume of extravascular fluid increases and, since it opposes the filtering pressure within the capillaries, makes itself evident in the diminished rates of filtration produced by any given venous pressure (Figures 6, 7 and 8)

The tissue pressure developed by the accumulation of small amounts of

fluid in the normal forearm can be estimated indirectly by observing the amount by which filtration is reduced When filtration is measured through periods of ten minutes, a change in venous pressure of 10 cm water modified the rate of filtration by 033 cc per minute per 100 cc of arm (Figure 3) As shown in Figure 8 when the filtered fluid amounted to 1 0 cc. per 100 cc of arm, filtration was decreased approximately 033 cc. per minute per 100 cc. of arm—a change which corresponds to the effect of lowering venous pressure by 10 cm water When the filtered fluid amounted to 5 0 cc per 100 cc. of arm the filtration was reduced by approximately 110 cc. per minute per 100 cc of arm—a change which corresponds to the effect of lowering venous pressure by 35 cm water It is obvious that absolute figures for tissue pressure thus indirectly obtained cannot have great significance until they have been verified by some more direct method

The first and apparently the only attempt to estimate tissue pressure directly was that of Landerer (16), who introduced a fine needle or cannula into the cutaneous and subcutaneous tissues in order to determine the pressure which was required to force small amounts of fluid slowly into the tissue spaces He concluded that in rabbits the tissue pressure is normally about 5 to 7 cm water, but varies widely in different tissues He found also that venous congestion elevated tissue pressure conspicuously, but mentioned neither the duration nor the grade of the venous congestion Various measurements of skin tension have been made by means of the elastometer (Schade (17), Kunde (18)) but these provide no information concerning absolute tissue pressures

It would appear, however that in the human being tissue pressure may be one of the most important factors in maintaining normal blood volume against considerable hydrostatic disadvantage Adolph (19) has suggested that in the observations of Krogh, Landis and Turner (1) venous pressures below 17 cm water failed to produce measurable filtration because of tissue pressure Thompson, Thompson and Dailey (20) and Waterfield (21) observed that blood volume decreased rapidly during the first 30 minutes of quiet standing after which period no further change in blood volume could be observed Reference to Figure 6 will show that it is during the first 30 minutes of venous congestion that the greatest decrease in filtration occurs

The physiological significance of this mechanism is obvious One of the greatest difficulties in applying the Starling hypothesis to problems of fluid balance in man has been the absence of local edema in the dependent parts of normal individuals In the lower extremities the excess of venous and capillary pressure over colloid osmotic pressure should, if Starling's two factors were the only ones concerned produce a considerable grade of local ankle edema. During quiet standing fluid is lost from the blood as shown by Thompson, Thompson and Dailey (20) but the loss ceases before edema appears

The dangers of reducing blood volume too greatly are obvious Quiet standing brings the vascular system quite near to complete failure and resultant syncope as shown by Turner, Newton and Haynes (22) and by Hamilton, Lichty and Pitts (23) Any mechanism which limits the amount of fluid which is lost to the tissue spaces will significantly reduce the need for cardiovascular readjustments in prolonged standing

The occurrence of edema shows in itself, however, that the power of tissues to resist the accumulation of tissue fluid is a limited one Underer believed that all forms of edema could be explained by the diminished elasticity of the edematous tissue But in determining the modulus of normal and edematous tissues he failed to take into the account that a given mass of edematous tissue contains fewer connective tissue fibers and more fluid than the same volume of normal tissue It would be expected, therefore, that under a given force edematous tissue would stretch more Bönninger (24), in testing the elasticity of the skin in cadavers, found no evidence that the elasticity of tissue was diminished in edema The skin removed from an edematous extremity shortened more than did skin taken from a normal extremity Bonninger found, however, that the tissues are very imperfectly elastic and when stretched even slightly they gradually elongate, failing to return to their original length when tension is removed Therefore, a relatively low tissue pressure acting for a long time might gradually stretch the tissue, the elasticity of which could delay, but could not prevent, the appearance of edema

No measurements of tissue pressure in edema are available The tightness of the skin in massive edemas suggests that the tissue pressure may be quite high under certain conditions It is a clinical truism that certain forms of edema appear first in the loose tissues of the orbit and face Yet the looseness of tissue is not the primary cause for the appearance of the edema, since, as Fishberg remarks (25), the relaxed abdominal skin of multiparae is not usually edematous

In considering the movement of fluid through the capillary wall the factors of first importance are capillary pressure, the colloid osmotic pressure of the blood, and the permeability of the capillary wall Two important subsidiary physical factors, temperature and tissue pressure, must be kept in mind since they can modify the effects produced by a given difference between capillary pressure and colloid osmotic pressure

SUMMARY

A pressure plethysmograph was arranged so that each determination of "reduced arm volume" (i e tissue volume with the blood vessels collapsed) required only two minutes' stoppage of blood flow The apparatus could be kept at a temperature constant within 1° C It was shown that measurements of "reduced arm volume" were relatively independent of the variations in arm volume produced by vasomotor

changes or by simple engorgement of the veins During two minute periods of 200 mm Hg pressure the plethysmograph expressed only negligible quantities of fluid from the segment of forearm enclosed within it

The movement of fluid through the human capillary wall was studied in relation to venous pressure, temperature and duration of venous congestion It was found that above an average venous pressure of 12 cm water the rate of filtration was directly proportional to the increase in venous pressure A unit rise in venous pressure (1 cm water) increased the filtration rate by 0028 cc. per minute per 100 cc of forearm when the congestion periods were 30 minutes long and by 0033 cc per minute per 100 cc of forearm when the congestion periods were 10 minutes long

The temperature of the forearm exerted a conspicuous effect on the rates of filtration produced by given venous pressures In general, the rates of filtration produced by venous pressures of 30 40, 50 and 60 cm water with a forearm temperature of 44 to 45° C were almost twice as great as those produced by the same pressures with a forearm temperature of 14 to 15° C

The rate of filtration produced by any given venous pressure decreased rapidly as fluid accumulated in the tissue spaces The filtration rate was reduced most rapidly during the first 30 minutes of venous congestion When sufficient fluid had accumulated in the tissue spaces low venous pressures failed to produce further filtration With large accumulations of fluid in the tissue spaces the filtration rate was decreased by an amount which was equivalent to a tissue pressure as high as 35 cm water

The importance of these two factors, temperature and tissue pressure, is briefly discussed with reference to normal fluid balance and to the formation of edema

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A NOTE ON CUTANEOUS VENOUS BLOOD SUGAR DIFFERENCE IN NORMAL MALES AND FEMALES AND IN THYROID DISEASE

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It has been well known since the publications of Foster (1) and of Hagedorn (2) that cutaneous blood obtained from a deep finger prick approximates arterial blood rather than venous blood in sugar concentration The deeper the prick and the more freely the blood flows from the finger the more closely the cutaneous values approximate arterial values

TABLE 1

Arterial, cutaneous and venous blood sugar values one hour after administration of 100 grams of glucose orally or 1 cc of 1 1000 epinephrine subcutaneously

	Arterial	Venous	Difference arterial-cutaneous
	mgm. per 100 cc	mgm. per 100 cc	mgm per 100 cc
After glucose	270	248	−3
	160	108	2
	149	118	2
	160	123	14
After epinephrine	165	157	−2
	142	134	1
	172	146	14

Table 1 shows the comparison of arterial, cutaneous and venous blood sugar values with our technique one hour after ingestion of 100 grams of glucose or after injection of 1 cc of 1 1000 epinephrine in subjects without evidence of circulatory or metabolic disorder Arterial blood was obtained from a brachial artery Cutaneous blood was taken from a finger tip following a puncture with a pen point deep enough to give a free flow Venous blood was drawn from the arm with only slight transient compression of the vein Blood sugar was measured by the method of Hagedorn and Jensen (3) In two out of seven comparisons considerable discrepancy is noted between arterial and cutaneous values. The sugar content of cutaneous blood is not in our hands a reliable

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measure of that of arterial blood in individual determinations, although tending toward agreement with it in most instances. In the course of measurements of blood sugar concentration in cutaneous and venous blood at ½, 1 and 1½ hours following ingestion of 100 grams of glucose by normal subjects we have noted a tendency toward higher cutaneous-venous differences in the females than in the males at the crest of the sugar curve.

TABLE 2

Cutaneous blood sugar value and cutaneous venous difference after ingestion of 100 grams of glucose at the examination showing highest sugar value

	Males			Females	
	Cutaneous	Difference cutaneous venous		Cutaneous	Difference cutaneous venous
	mgm. per 100 cc.	*mgm. per 100 cc.*		*mgm. per 100 cc.*	*mgm. per 100 cc.*
Normals	198	48	Normals	203	61
	211	32		135	42
	173	26		135	39
	159	25		137	38
	148	24		144	36
	182	22		135	33
	190	20		172	32
	179	18		201	31
	182	13		124	28
Hyperthyroid	281	49	Hyperthyroid	174	49
	239	0		219	37
				355	30
				262	30
				133	27
				217	27
				230	22
				274	21
				232	15
				234	10
				189	−4
			Hypothyroid	223	89
				206	80
				171	49
				220	46
				181	40

This is shown in Table 2. It includes as "normals" the values on 9 male and 9 female medical students or members of the laboratory staff who, with the exception of one male who was 40, varied in age between 18

and 30 The cutaneous and venous blood, taken fasting contained nearly the same concentration of sugar The cutaneous-venous difference became definite as the blood sugar rose The tendency toward greater cutaneous venous difference at the crest of the curve in the females than in the males was associated with lower rather than higher sugar values in cutaneous and venous blood in the females

In Table 2 are shown also the cutaneous venous differences at the crest of the curve following ingestion of 100 grams of glucose in 11 female and 2 male patients with hyperthyroidism and in 5 females with hypothyroidism In about half the patients with hyperthyroidism the cutaneous-venous differences are lower than normal In most of the hypothyroid patients the cutaneous-venous difference is high No consistent difference in the cutaneous sugar values is to be noted in the two groups

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